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Complexation of amino-acid derivatives using macrocyclic receptors

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Complexation Of Amino-Acid Derivatives Using Macrocyclic Receptors

submitted by **David John Smith**

for the degree of PhD of the University of Bath

1996

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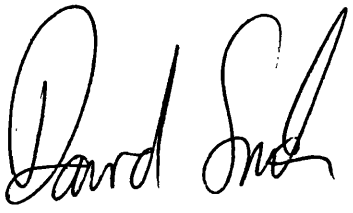

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To Sarah

The man who does not read good books
has no advantage over the man who can't read them

Mark Twain

Acknowledgements

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Many thanks to my former flat-mates Dr John "you'll have another" McGinley and Dr Kim "that will never work" Wong; Dr Jackie " Haggis" McInnes, a former new kid on the block, and Dr David "Gunner" Evans for all their support and humour since October 1992.

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Finally, many thanks to my wife, Sarah, whose encouragement and support during the past three years, through the highs and lows of PhD study, has made this thesis possible.

Abstract

The aim of this project was to investigate the potential of a series of oxa- and aza-crown ethers to act as hosts for specific organic acids and amino-acids, so leading to the removal, or deactivation via complexation, of minor organic contaminants present in fermentation media. The main target guest compounds in this study were 4-aminobutan-1-oic acid (GABA) and glutamic acid, and 18-crown-6 and 15-crown-5 receptors were chosen as hosts because of their known ability to complex primary amine derivatives, and because they can be readily chemically modified in order to both change their selectivity and facilitate their attachment to a support.

Chapter One contains a general account of host-guest chemistry, starting with 2,2'-bipyridine and including specific natural compounds before considering more fully the role of crown ethers as metal ion and organic compound receptors. A review of the synthesis and structural modification of crown ethers is followed by a consideration of siloxane polymer attachment methodology. The use of fluid organofunctional siloxanes as separants, and relevant examples of crown ether functionalised siloxane polymers are then described.

Chapter Two summarises all the experimental methods employed during this investigation. The chapter is divided into three basic sections containing details of:-

i) the synthesis of 28 crown ether analogues, as depicted on pages xvi-xviii, including the preparation of 19 novel receptors based on monoaza-15-crown-5 and monoaza-18-crown-6 ranging from mono- and di-substituted alkenyl derivatives to bis(monoaza-18-crown-6) compounds.

ii) the attachment of 1-hexenyl-monoaza-15-crown-5 and 1-butenyl-monoaza-18-crown-6 to 1,1,1,3,3,5,5-heptamethylhydridotrisiloxane, and 1-butenyl-monoaza-15-crown-5 and 1-hexenyl-15-crown-5 to 1,1,1,3,5,5,5-heptamethylhydridotrisiloxane and the tethering of 1-hexenyl-15-crown-5, 1-butenyl-monoaza-15-crown-5 and 1-hexenyl-monoaza-15-crown-5 to a (3-4%)-methylhydro-(96-97%)-dimethylsiloxane copolymer shown on page xviii.

iii) several miscellaneous reactions which have been important to the overall development and direction of this work.

Analytical data for the products are given after each description of the preparative route.

Chapter Three contains an account of the analytical techniques used in the characterisation of the compounds described in **Chapter Two**. A general introduction is given to each analytical method, the key features of the technique, and its application in the characterisation of the respective compounds, beginning with infra-red spectroscopy, followed by nuclear magnetic resonance spectroscopy, including ^{1}H, ^{13}C, ^{29}Si and COSY n.m.r. spectroscopy, instrumental elemental analysis, and then mass spectrometry. Finally, the use of high performance liquid chromatography as an analytical tool, and the method employed in this investigation, is described. Preliminary attempts to extract glutamic acid using a butenyl-monoaza-15-crown-5 functionalised (3-4%)-methylhydro-(96-97%)-dimethylpolysiloxane copolymer from aqueous solution is also reported.

Chapter Four begins with an assessment of the structural features of GABA and glutamic acid and the types of interaction which may occur between each compound and a prospective receptor. Specific examples of synthetic host compounds which have been applied to the recognition of amino-acids and their derivatives are then highlighted. The

experimental development of the various crown ether based receptors used in this study, starting with simple mono-functionalised oxa- and aza-crown ethers and ending with the more complex bis(monoaza-18-crown-6) compounds, is then described. H-H COSY n.m.r. spectroscopy was used to determine the effectiveness of 11 crown ether compounds to act as receptors for GABA and glutamic acid by analysing 1:1 mixtures of the host crown ether derivative and the guest acid under various pH conditions. The chapter ends with a discussion of proton chemical shift data and their implications, conclusions and recommendations for future work..

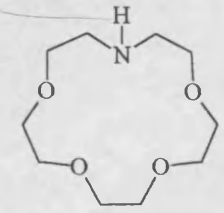
Contents

Abbreviations

Å	Ångstrom
acac	2,4-Pentanedionate anion
Ar	Aromatic
asym	Asymmetric
br	Broad
^{t}BuOH	2-Methyl-propan-1-ol
C.I	Chemical ionisation
COSY	Correlated spectroscopy
d	Doublet
DCC	1,3-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DCU	Dicyclohexylurea
DEPT	Distortionless enhancement by polarisation transfer
DMF	N,N-dimethylformamide
DMSO	Dimethylsulphoxide
E.I..	Electron ionisation
FAB	Fast atom bombardment
FCP	Functionalised polysiloxane
FMOC	9-Fluorenylmethylchloroformate
fod	6,6,7,7,8,8,8-Heptafluoro-2,2-dimethyloctane-3,5-dionate anion
GABA	4-Aminobutan-1-ioc acid
GC	Gas Chromatography
Glu	Glutaric acid
HMDSO	Hexamethyldisiloxane

HOBT	Hydroxybenzotriazole hydrate
HPLC	High performance liquid chromatography
I	Spin isotope
i.r.	Infra-red spectroscopy
m	Multiplet
m/e	Mass to charge ratio
MeCN	Acetonitrile
MeOH	Methanol
MHz	Megahertz
MMG	*mono*-Methyl glutarate
mmHg	Millimetres of mercury
n.m.r.	Nuclear magnetic resonance spectroscopy
NOE	Nuclear Overhauser effect
o	Ortho
oct	Octet
ODS	Octadecylsilane
OPA	o-Phthalaldehyde
p	Pentet
pm	Picometres
ppm	Parts per million
q	Quartet
R	Alkyl
s	Singlet
str	Stretch
sx	Sextet

sym	Symmetric
t	Triplet
TEAH	Tetraethylammonium hydroxide
TFMSA	Trifluoromethane sulphonic acid
THF	Terahydrofuran
TMS	Tetramethylsilane
Tosylate (Ts)	*p*-Toluenesulphonyl, *p*-$CH_3C_6H_4SO_2$-
Val	Valeric acid
12-crown-4	1,4,7,10-tetraoxacyclododecane
15-crown-5	1,4,7,10,13-pentaoxacyclopentadecane
18-crown-6	1,4,7,10,13,16-hexaoxacyclooctadecane



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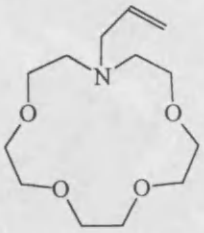

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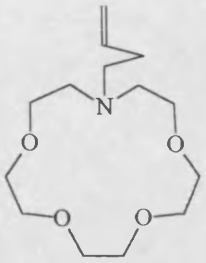

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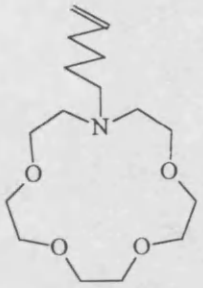

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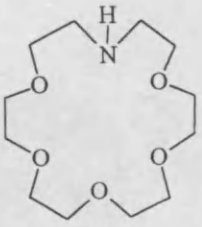

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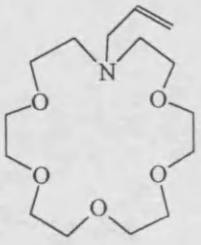

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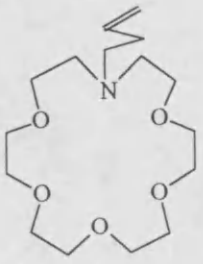

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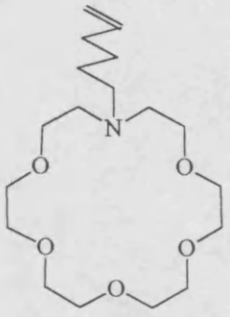

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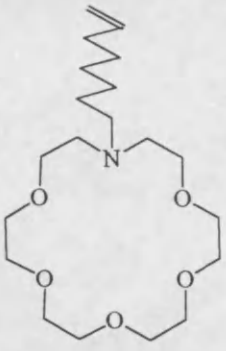

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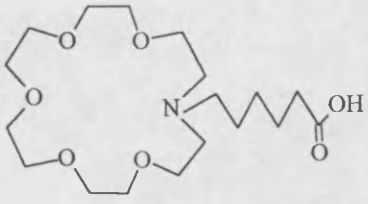

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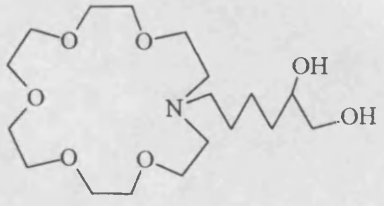

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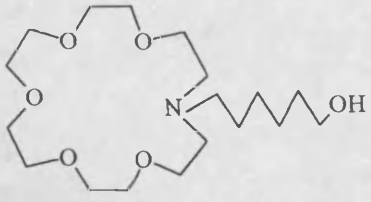

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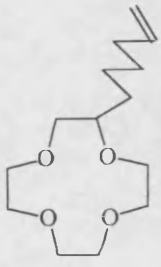

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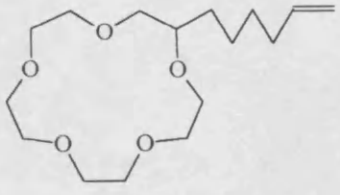

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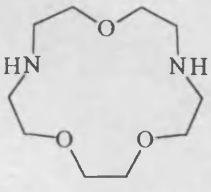

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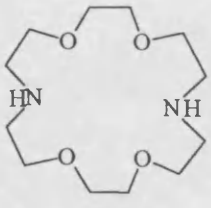

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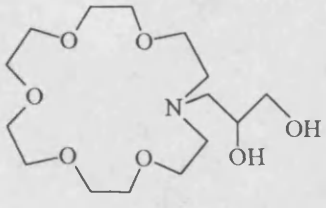

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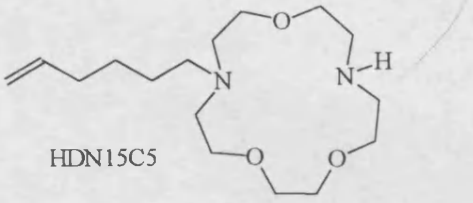

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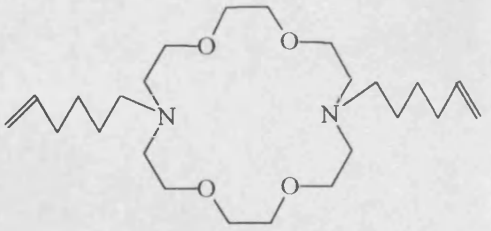

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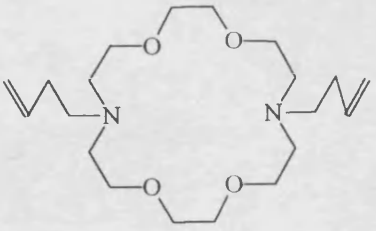

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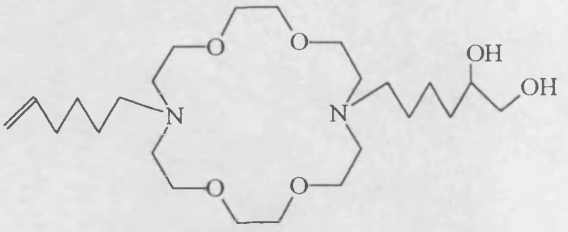

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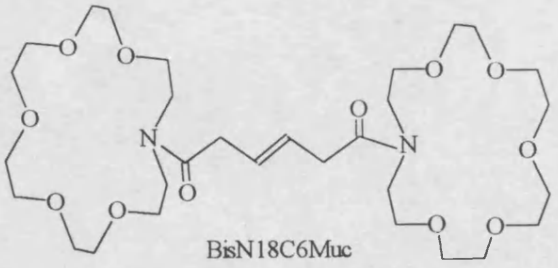

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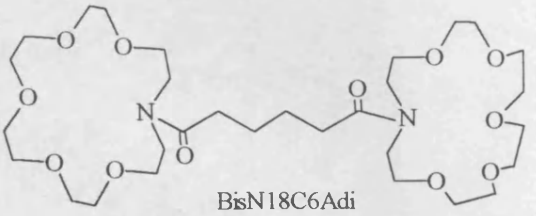

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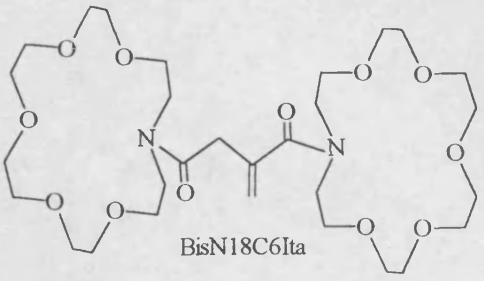

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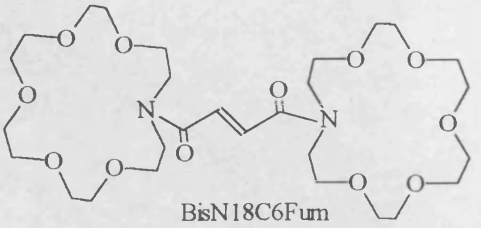

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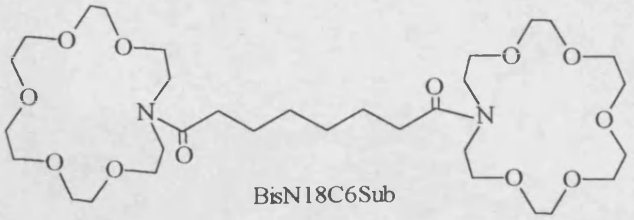

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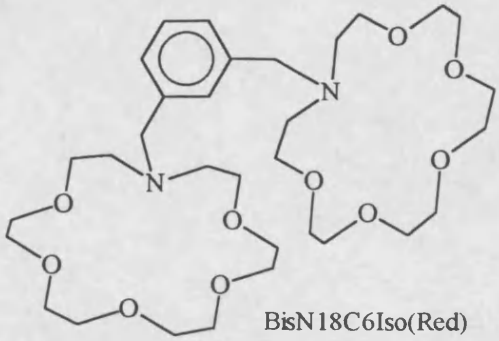

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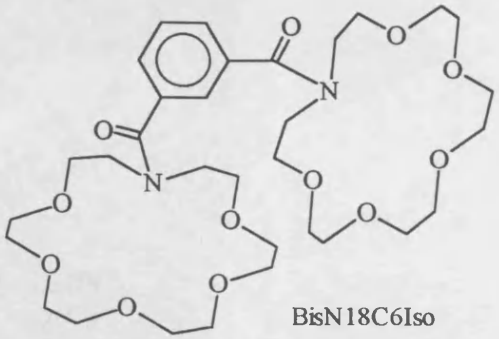

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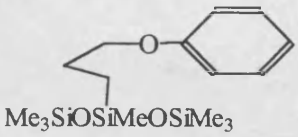

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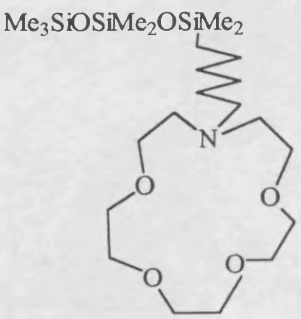

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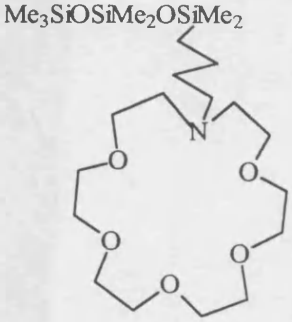

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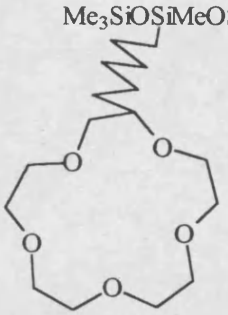

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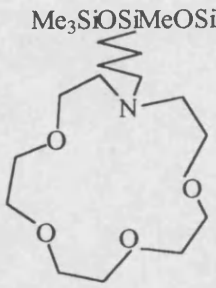

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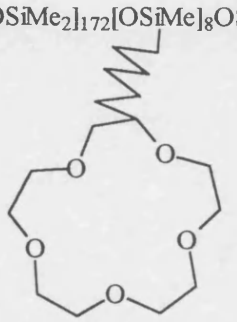

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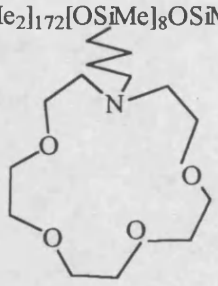

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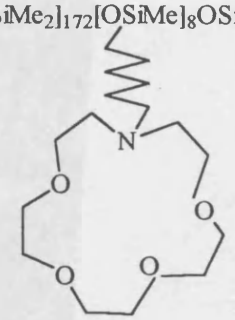


HN15C5PS

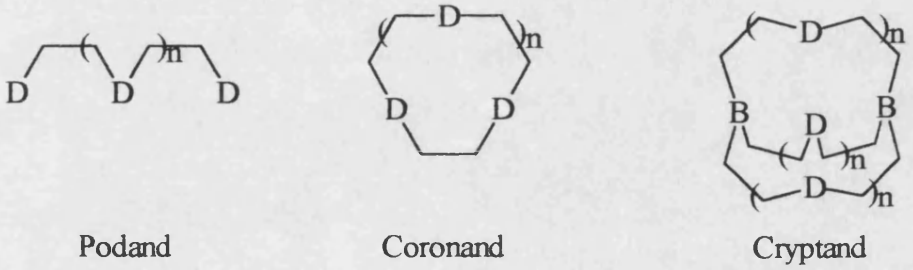
Chapter One

Introduction

1. Host-Guest Chemistry

1.1. Introduction

Host-guest chemistry, also known as molecular recognition chemistry, is a wide, varied, and highly active subject which encompasses the synthesis and study of new receptor molecules as well as the adaptation of natural compounds for use in biomimetic studies[1-2]. In its simplest form it is concerned with the recognition and binding of a guest species by a natural or synthetic host or receptor[3], and its expansion and applications have relied heavily on an understanding of biological processes, on the development of the chemistry of acyclic receptors (podands), and more particularly on advances in the host-guest chemistry of crown ethers (coronands) and their cyclic derivatives such as cryptands (cavitands)[4-6] (**Figure 1**).



Podand Coronand Cryptand

D = Donor B = Bridgehead atom

Figure 1

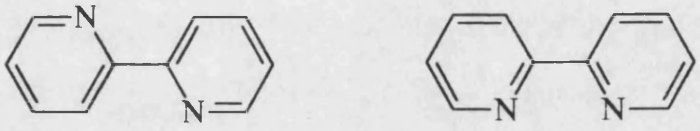
Complementary non-covalent interactions between the host and the guest are often of fundamental importance if binding of a specific substrate type is to be achieved, and these are frequently size or shape specific. Interactions present in host-guest complexes vary from relatively strong, directional, co-ordinate linkages through to hydrogen-bonding, ion-pairing, π-π binding dispersion interactions, van der Waals attractions and constructive solvent effects[7].

The potential binding sites of the host may be presented in the correct orientation and readily accessible to the guest continuously. Compounds with organised binding sites are described as preorganised hosts. Alternatively the disposition of the binding sites may change on introduction of the guest, so giving more effective interactions as the guest is introduced[8].

1.2. Host Compounds and their Complexes

1.2.1. 2,2'-Bipyridine

One of the earliest and most studied ligating species which can be considered to be a host compound in the widest interpretation of this term is 2,2'-bipyridine (**Figure 2a**).



(a) (b)

Figure 2

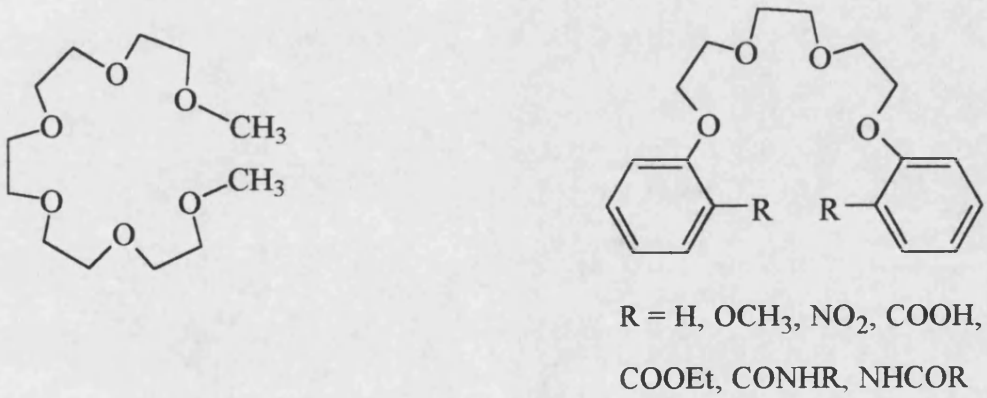
In the presence of a metal ion it changes its free state conformation from that shown in **Figure 2a,** to that in **Figure 2b** and then acts as an excellent host species for a large range of neutral and charged metal ions. Co-ordination occurs via strong directional σ-bonds involving the N-donor centres[9] and results in the formation of a stable, relatively strain-free 5-membered chelate ring.

Bipyridine has been widely used in supramolecular receptors because it can be easily modified. It provides the basis for an extensive series of acyclic polypyridine ligands for example, which exhibit interesting and extensive host properties, including the formation of double helices around a number of metallic guest centres[10].

Hosts of this type frequently form very strong complexes with metal ions, and hence they are often not suitable for application in reversible separation procedures. Other hosts with weaker and preferably reversible interactions with a guest are of more relevance therefore for the recognition and separation of guests.

1.2.2. Podands

Podands are open chain receptor compounds which have donor groups at one or both ends and at least one donor centre situated within the chain. The podand group of receptors are typified by the oligomeric polypyridines described above, or by glycols which feature an all oxygen donor set and are generally classified as acyclic crown ethers[11] (**Figure 3**).



R = H, OCH_3, NO_2, COOH, COOEt, CONHR, NHCOR

Figure 3

The $-CH_2-CH_2-O-$ moiety is a common building block found in many acyclic and cyclic polyethers because of the strong chelating effect from electron lone-pair donation from two oxygen centres on incorporation of a metal ion, leading to strain-free 5-membered rings. The flexibility of the $-CH_2-CH_2-$ spacer chain compared with a rigid aromatic backbone (e.g. as in polypyridine) confers a greater degree of tolerance, and hence less specificity, to receptors containing this unit.

The synthesis of podand type receptors with specific terminal groups has led to the development of some important materials[12], an example of which is Kryptofix-5® (**Figure 4**), which is able to wrap around the host substrate so imparting increased lipophilicity and facilitating dissolution in organic media[13].

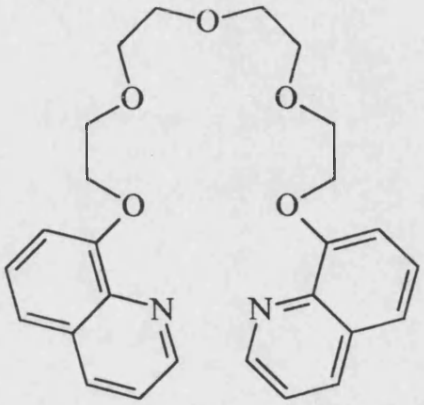


Figure 4

Nabeshima and co-workers[14] have used this encapsulation approach to selectively bind Cu(I) and Ag(I) in a bipyridine terminated polyether and polythioether respectively. The host compound which they report, and Kryptofix-5®, both undergo structural changes on the introduction of the respective cation guest, resulting in allosteric effects which change the host's ability to bind a further metal cation guest. In the example below the allosteric effects are constructive and allow the host compound to bind a second metal cation more effectively (**Figure 5**).

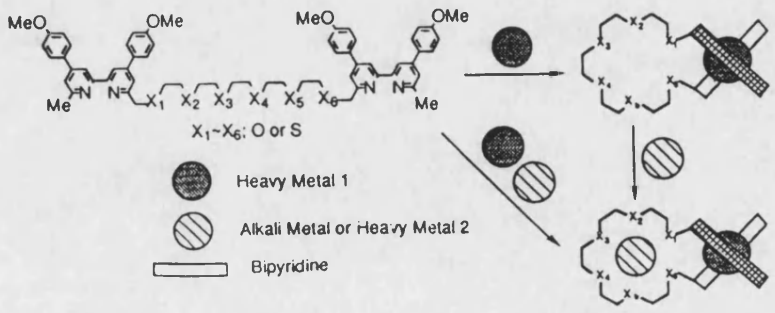


Figure 5

Solvent extraction studies showed that Cu(I) polyether complexes did not extract Ni(II), Pb(II), Mn(II) and Co(II) to any degree, although Ag(I) was selectively extracted.

However, allosteric effects can be such that in a host compound with two possible binding sites the inclusion of one of the guests results in a host conformation which is unable to accommodate a further guest[15-16]. Chambron and Sauvage[17] synthesised a ligand which contained two co-ordination sites with different selectivities. Ruthenium(II) was bound in an octahedral environment allowing Cu(I) to be complexed in a tetrahedral configuration. Electron-transfer studies on photo- and electro-active centres within the complex were conducted giving an insight into redox processes of relevance to photosynthesis.

1.2.3. Coronands and Cryptands

The chemistry of synthetic macrocyclic receptors originates with Pedersen's discovery of crown ethers in 1962[18], and since then crown ethers and cryptands, bi- and tricyclic crown ethers (**Figure 6**) have been the centre of much attention[19]. The far-reaching consequences of Pedersen's discovery were recognised in 1987 when he, together with two other pioneers in this field, namely Lehn and Cram, were awarded the Nobel Prize for chemistry.

Figure 6

The first crown ether, dibenzo-18-crown-6 (DB18C6) (**Figure 7**), was discovered by accident as a by-product in the preparation of bis[(2-o-hydroxyphenoxy)ethyl]ether. Due to the presence of unprotected catechol, equimolar quantities of the phenol derivative and dichlorodiethyl ether present in the reaction mixture underwent a cyclisation reaction, so forming the crown ether in 0.4% yield.

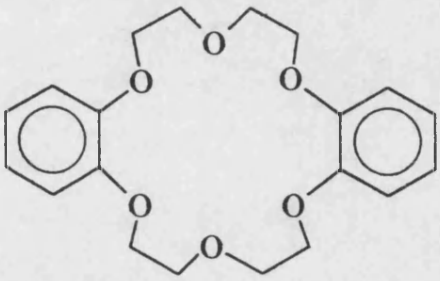


Figure 7

The ability of this new compound to form stable lipophilic complexes, via lone pair donation with alkali- and alkaline earth metals, an example of which is shown in **Figure 8**[20], led to the eventual preparation of an extremely large family of crown ether species which have been utilised for many different purposes.

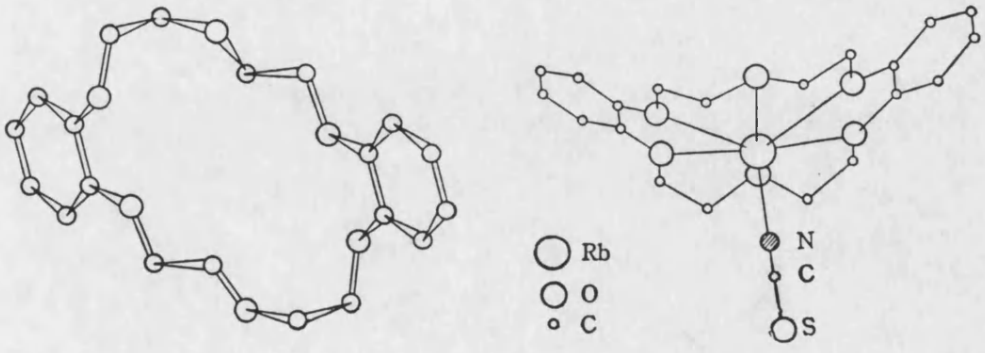


Figure 8

The uses of crown ethers can be loosely classified as dependent upon:- (A) the capacity of crown ethers to selectively capture, separate and transport cations; and (B) their ability to solubilise inorganic species in organic solvents[21]:-

A) Applications of crown ether compounds utilising their capacity for selective capture, separation and transport of cations include:-

(i) Separation or concentration of specific alkali or alkaline earth metal cations from mixtures, e.g. Na^{+}-K^{+} and Ca^{2+}-Sr^{2+} from each other.

(ii) Capture, recovery, removal, separation, concentration, or purification of heavy metal ions such as Ag^{+}, Cu^{2+}, Hg^{2+} and Pb^{2+}.

(iii) Separation of individual lanthanides from solution.

(iv) Ion-selective electrode detection of alkali-, alkaline-earth and lanthanide metal ions.

(v) Ion transport using crown ether functionalised membranes including liquid membranes.

(vi) Optical resolution using optically active crown ether compounds as hosts.

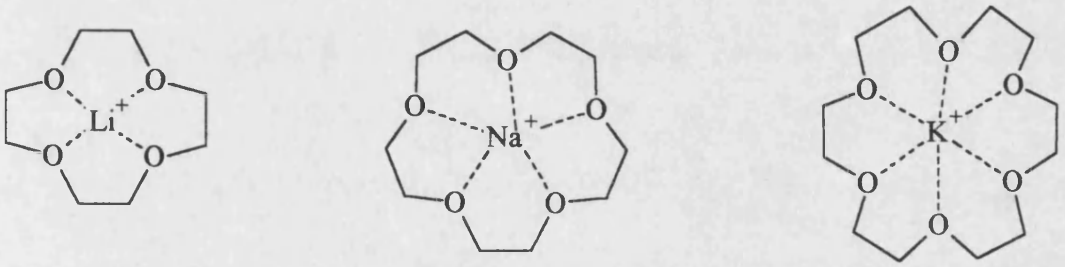
B) Applications related to their ability to solubilise inorganic species in organic solvents and either activate or stabilise them include:-

(i) Inorganic syntheses including the formation and stabilisation of metal carbonylate anions.

(ii) Organic syntheses in which reactions proceed under milder conditions than otherwise, due to the increased activity of a "naked" anion.

(iii) Analytical applications in which chiral crown ethers bound to stationary phases can be used to effect chiral resolutions using HPLC or GC techniques.

As crown ethers have a central cavity into which a metal cation can be held electrostatically by ion-dipole interactions, the size, flexibility and spacer chain length between the donor centres of the crown ether determine the size of the cavity. Therefore different cations can be selectively complexed to some degree by specific crown ethers[22]. Thus 12-crown-4 (12C4) forms strong complexes with Li^+, 15C5 prefers Na^+ and 18C6 will preferentially bind K^+ (**Figure 9**; **Table 1.1**).



12C4 15C5 18C6

Figure 9

Cation	Diameter(pm)	Crown	Diameter (pm)
Li^+	136	12C4	120-150
Na^+	190	15C5	170-220
K^+	266	18C6	260-320
Cs^+	338	21C7	340-430
a		24C8	>400

[a]More than one metal cation may be incorporated in rings of this or larger sizes

Table 1.1 Comparison of the Diameters of Different Alkali Metal Cations and Crown Ether Cavity Sizes.

Crown ethers are not completely ion specific, and are sometimes able to bind effectively ions which do not match the central cavity of the crown ether. For example a large cation can be accommodated "on top" of a crown ether into which it will not fit, or it can be sandwiched between two crown ethers[23] (**Figure 10**).

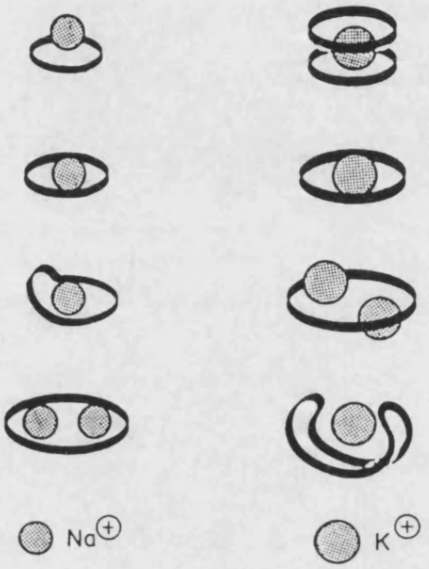


Figure 10

Quantification of the ionophoric behaviour of crown ethers quickly followed their discovery[24-26], and formation constants for 1:1 metal cation complexes in a variety of solvents have been accurately determined[27]. Selected data for dicyclohexyl-18-crown-6 and alkali and alkaline earth cations are highlighted in **Table 1.2**.

Solvent	Metal Ion	Formation Constant (Log K_f)
H_2O	Na^+	1.50
H_2O	K^+	2.18
H_2O	Ba^{2+}	3.44
H_2O	Sr^{2+}	2.80
MeOH	Na^+	4.09
MeOH	K^+	5.89

$$K_f = \frac{[\text{Host / Guest}]}{[\text{Host}]\,[\text{Guest}]}$$

[ ] = Concentration of substrate

Table 1.2 Potentiometrically Determined Formation Constants of Dicyclohexyl-18-crown-6 Complexes with Alkali- and Alkaline Earth Metal Ions in Solution.

From **Table 1.2** it can be seen that the degree of complexation is affected by three factors. These being the size of the macrocyclic host relative to that of the guest, the charge of the ionic species being complexed, and the medium in which the components are dissolved. It is important to note in the context of this study that the complexation of cations by dicyclohexyl-18-crown-6 is considerably less in a solvent of higher donicity. For example, in aqueous solutions metal cations are highly solvated by co-ordinated water molecules and Iwachido et al[28] have shown that on complexation a metal guest cation may retain some of its aqueous solvation shell. This is particularly important for small monovalent cations, such as Li+ and Na+, and for divalent cations, all of which exhibit high hydration numbers for the respective crown ether complexes[29].

There are now many crown ether analogues in which one or more of the oxygen donors have been replaced with a nitrogen or sulphur containing sub-unit, resulting in an aza- or a thio-crown ether respectively as illustrated below (**Figure 11**).

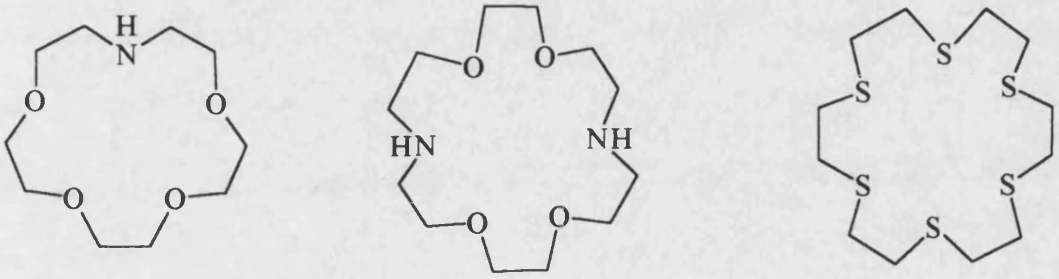


Figure 11

The presence of the different heteroatoms affects the complexing ability of the crown ether. Oxygen centres are deemed to be hard donors and will therefore preferentially produce a more strongly binding ion-pair (large K_f) with non-polarisable hard metal ions, such as those formed by potassium and sodium. Conversely, sulphur is classed as a soft donor and will preferentially form ion-pairs with polarisable transition metal centres. Nitrogen containing crown ethers are intermediate in behaviour as noted in **Table 1.3**.

		Formation Constants (Log K_f)		
Metal Ion	Solvent	18C6	1,7-Diaza-18C6	1,7-Dithia-18C6
K^+	MeOH	5.89	2.04	1.15
Ag^+	H_2O	1.70	7.80	4.34

Table 1.3. Effect of Donor Atoms on the Complex Formation Constants (Log K_f).

The presence of one or more NH groups in an aza-crown ether confers an additional benefit in permitting the facile functionalisation of the receptor after the macrocycle has been formed. Consequently di- and tri-aza crown ethers are frequently used as building blocks for more complex analogues such as cryptands[30-32]. For oxygen and sulphur analogues any additional functionality must be introduced onto a carbon atom, and hence it is more appropriately introduced, usually with some difficulty, prior to cyclisation.

It should be noted that due to their complexing ability on biologically important metals, including the alkali metals, crown ethers and their derivatives are, to a greater or lesser extent, toxic which needs to be considered when working with them or deciding upon their applications[33].

1.2.4. More Complex Host Species

As the host compounds become more preorganised, in the series podands, cyclics (crown ethers) and polycyclics (cryptands), they are able to complex more strongly with metal ions, so resulting in increased formation constants (K_f)[34]. Crown ethers and cryptands exhibit the macrocyclic and the macrobicyclic effect respectively on forming metal complexes. The former occurs where prospective non-covalent bond forming atoms, such as oxygen or nitrogen, are preorganised, often in an approximately 2-dimensional arrangement, so as to maximise the host-guest interactions[35-36]. The latter also has preorganised donor sites, but provides a 3-dimensional environment for the guest, so encapsulating it completely. The stabilities of the host-guest pairs result from enthalpic contributions from the interactions between the donor sites of the crown ether and the metal ion, and from entropic contributions due to the formation of chelate ring systems.

Typical values for the formation constant (K_f) between podands, crown ethers and cryptands, all having comparable hard donor centres, with alkali and alkaline earth metal cations dissolved in methanol are given in **Table 1.4**.

Type of Host	Type of Complex	K_f	Stabilising Effect
Podand	Podate	10^2-10^4	Chelate
Coronand	Coronate	10^4-10^6	Macrocyclic
Cryptand	Cryptate	10^6-10^8	Macrobicyclic

Table 1.4. Relationship Between the Host, Type of Complexation, Typical Complex Formation Constant (K_f) for Metal Cations, and the Stabilisation Effect Involved.

In this study crown ethers were chosen as the starting materials for the receptors since they can be modified to improve their binding / selectivity for target hosts, as shown in a recent study on specific metal cation targets[37]. The interaction of crown ethers with target guest species may be controlled by variation in pH and / or solvent composition. They can also be immobilised on a support if suitably modified.

It should be noted that there are a large number of synthetic host species which have more complex 3-D structures than coronands and cryptands. Examples are the calixarenes and the spherands as illustrated in **Figures 12** and **13** respectively.

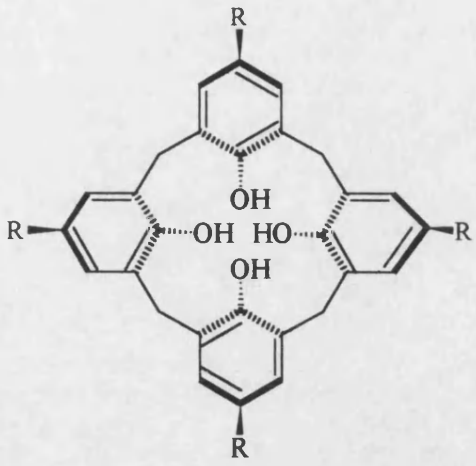


Figure 12

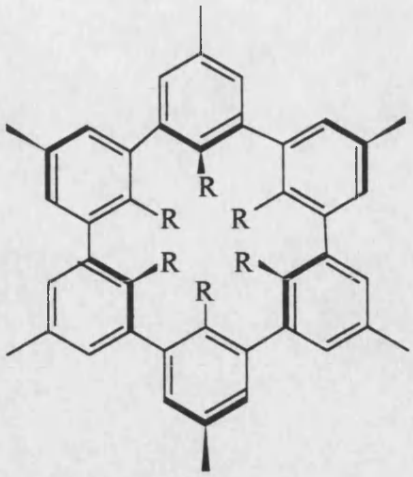


Figure 13

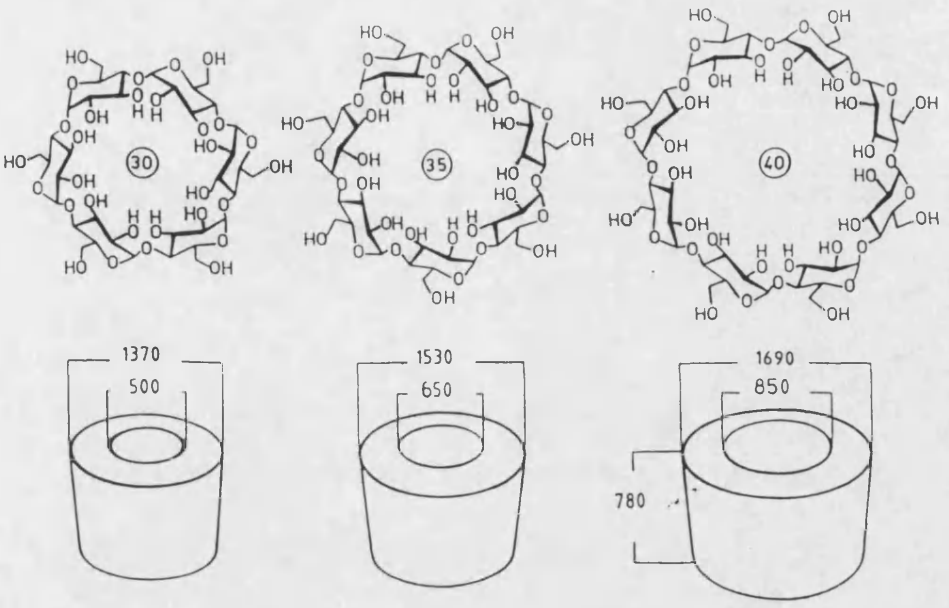
In calixarenes the R groups are typically methyl or t-butyl groups whilst in the spherands they contain the donor centres and are commonly hydroxyl or methoxy substituents. The donors of the calixarenes hang below the remainder of the molecule whilst the alkyl groups are situated at the "top" of the molecule completing a cup-like structure. However, the donor sites in spherands are part of the intraannular components and point inwards towards the rigid ring system resulting in them being alternately slightly above or below the horizontal plane of the molecule.

Calixarenes were originally obtained from the condensation of formaldehyde (methanal) and p-alkylphenols. This reaction results in the inclusion in the macrocycle of a flexible CH_2 linkage[38], which acts as a "joint" and gives the typical cup-like structure into which the guest fits and from which the name calixarene arises[39]. There are many other host compounds which have been reported, some of which are analogues of natural receptors found in biological systems, but due to the nature of this study they will not be considered in this introduction.

1.2.5. The Inclusion of Metal Cations into Natural Host Species

Crown ethers containing cationic metal ion guests, particularly those of the alkali-[40-42], alkaline earth metals[43-44] and the lanthanides[45], all of which are strongly electropositive, have been studied intensively in the last three decades. One reason for the recent interest in studying the binding of metal ions to synthetic hosts relates to modelling the transport of metal cations, especially those of sodium and potassium in biological systems[46]. Changes in the balance between the sodium and potassium ion concentration and transport through and around living cells in vivo causes adverse effects on the cells and their biological surroundings. The transport of sodium ions around the body is of vital importance for the correct functioning of the many different systems of the body, such as nerve and muscle fibres for the transmission of impulses, and also for the prevention of cellular swelling. Movement of sodium ions coupled with the counter-transportation of potassium cations, gives rise to what is commonly termed the "sodium pump". Energy from the sodium pump is necessary for the transport of amino-acids around the body if protein deficiency is to be avoided[47].

There are a number of natural receptors such as cyclodextrins and valinomycin, which are involved in the biological transport mechanisms referred to above[48], and many studies on trans-membrane transportation have been carried out in an attempt to gain a greater knowledge of the transport mechanisms which occur in biological systems[49-50]. Cyclodextrins are composed of glucose sub-units with pendant hydroxyl groups, and they have a range of ring sizes which confer specific complexing properties to each analogue for the transport of metal cations[51] (**Figure 14**).



Cyclodextrins: dimensions are in pm, with the corresponding ring sizes given in the centre of the rings.

Figure 14

Valinomycin (**Figure 15**)[52] is a cyclic peptide and has a primary structure constructed from amino-acids. A metal cation can be encapsulated by the flexible valinomycin backbone and bound by electrostatic interactions between the cation and six ester carbonyl oxygen atoms. The strength of the host-guest interactions is further enhanced by the stabilising effect of six intermolecular hydrogen bonds between all the -NH groups and the amide carbonyl oxygens[53].

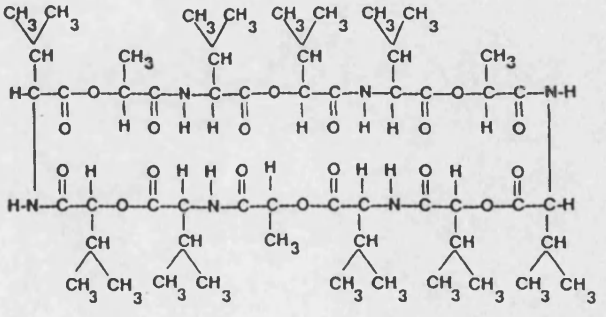


Figure 15

These two natural hosts typify the requirements of effective synthetic host species for the complexation of a specific guest molecule by having, or rearranging to form, a suitable cavity size into which the guest can fit and a number of correctly situated binding groups which are capable of constructively ligating the guest and holding it in place in the most appropriate environment[54].

1.2.6. The Binding of Water By Crown Ethers

Since the central cavity in crown ethers and other receptors is surrounded by highly polar functional groups, it is not surprising to find that such hosts will entrap small, polar, neutral and cationic species which can form hydrogen bonds. One or more water molecules can be easily incorporated via hydrogen bonding into a variety of crown ethers, independent of the size of the cavity. An example is illustrated below (**Figure 16**).

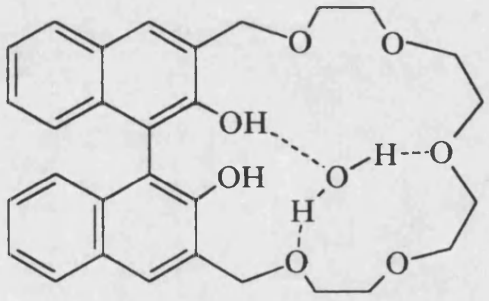


Figure 16

Willey and co-workers[55] demonstrated the co-operative effect of water in stabilising the binding of a metal ion. The crystal and molecular structure of the aza-crown ether-tin (IV) complex, $[H_2L]_2[SnCl_5(H_2O)]_2 \cdot H_2O \cdot MeCN$, where HL is mono-aza-18-crown-6, revealed that the two independent aza-crown ether molecules were both protonated. The co-ordinated water molecule was located over the centre

of one of the aza-crown ether moieties and was held by strong hydrogen bonds to the nitrogen atom (O_{water}-----H-N_{ring}, 2.690Å) and two oxygen atoms of the macrocycle (O-H_{water}-----O_{ring}, 2.793Å). Since cationic species are normally preferred by crown ethers to neutral molecules it is not surprising to find that H_3O^+ can also be stabilised in a suitable crown ether. A crystal structure determination of dicyclohexano-18-crown-6 containing H_3O^+ revealed three O----H-O^+ hydrogen bonds between the hydronium ion and the oxygens of the macrocycle with bond lengths around 1.7Å[56].

1.2.7. The Inclusion of Organic Species by Crown Ethers

The preorganised nature of 15-crown-5 and 18-crown-6 makes them well suited to act as receptors for other non-metallic cations which are electronically equivalent to H_3O^+, such as substituted ammonium cations[57-58]. A 1:1 crystal structure of *tertiary*-butylammonium : benzo-15-crown-5, revealed N-H----O hydrogen bond lengths in the region of 2Å, and a schematic diagram showing the host-guest intermolecular hydrogen bonding is provided in **Figure 17**.

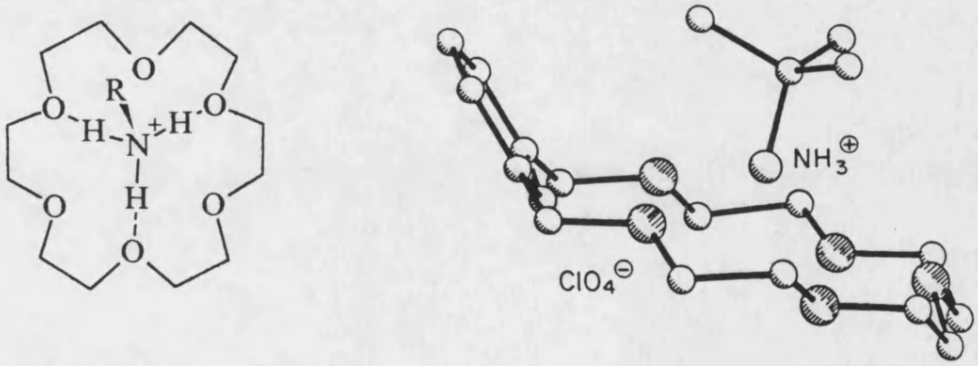
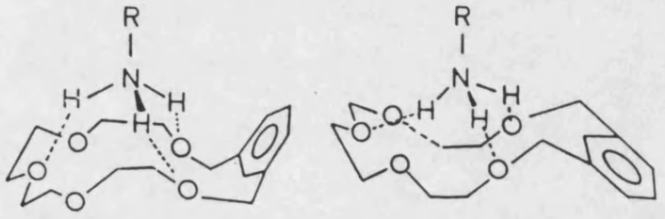


Figure 17

From crystallographic and molecular modelling studies, using the CPK (Corey-Pauling-Koltun) modelling system, there is evidence that the $-NH_3^+$ moiety of a protonated primary amine can form hydrogen bonds with a crown ether system in two ways , which depends on the conformation of the receptor. These are illustrated in **Figure 18 a** and **b** and are described as nesting and perching conformations respectively[59]. The perching conformation is the preferred configuration since the guest can be accommodated closer to the ring, resulting in shorter, stronger hydrogen bonds.



(a) **(b)**

Figure 18

Hosts which strongly bind more complex organic species usually incorporate three or more donor sites, arranged in a non-planar distribution. Work by Dumont et al[60] and Flack and co-workers[61] provide examples of such receptors involving the guests dopamine and dicarboxylic acid derivatives respectively (**Figure 19**).

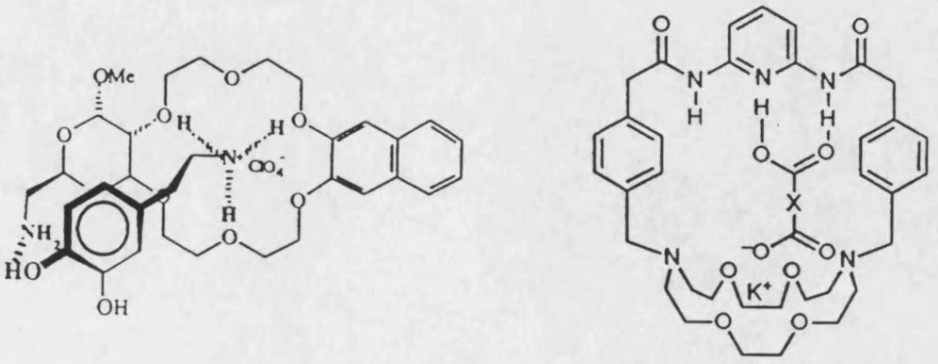


Figure 19

The abilities of host species to bind neutral and organic substrates are of growing interest[62-63] since this behaviour widens the range of possible applications of these host compounds, particularly in the fields of biology and medicine[64-65] as mentioned earlier. The binding and subsequent transport of the organic species, for example amino-acids and their derivatives[66], are of prime importance for the correct functioning of biotic systems, with L-dopa (L-dihydroxyphenylalanine), a dopamine receptor agonist used in the treatment of Parkinsons Disease, and 4-aminobutanoic acid (GABA), a synaptic mediator, necessary for the correct functioning of the brain and nervous system[67-68]. The range of organic compounds which have been complexed by crown ethers and their analogues vary from simple substituted ammonium species to more complicated molecules such as glycine, L-alanine, L-valine along with other amino-acids and organometallic compounds, for example ferrioxamine B, which influences the bioavailability of metals[69] (**Figure 20**). The use of synthetic compounds, with specific complexing abilities, to act as enzyme models has been extended to the development of novel chiral receptors[70].

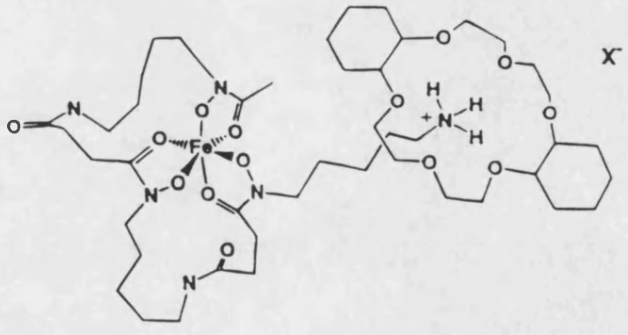


Figure 20

In order to obtain the moderately strong, but reversible, host-guest interactions which are necessary for an effective separation process, consideration must be given when designing the host to the relative stereochemistries of the host and the guest, in order to introduce the necessary structural complementarity[71]. For crown ether based receptors ring size is of primary importance, but additional functionalities may be necessary to improve selectivity.

1.2.8. Derivatisation of Crown Ethers to Form More Complex Receptors

Early in the development of crown ether chemistry more complex derivatives were developed which contain one or more side chains which can give additional interactions with the guest species ("lariat" side chains)[72-73]. Functional group attachments to both carbon and nitrogen atoms of macrocycles have been reported, and their chemistry has been reviewed by Krakowiak et al[74].

Other examples of this approach are described in a study by Voyer et al[75], in which two linked crown ether derivatives were used to encapsulate a range of diammonium guests, highlighting the effect of constructive co-operative binding (**Figure 21**).

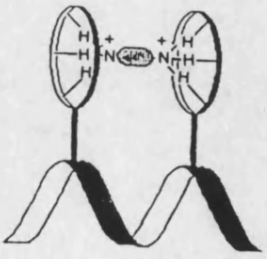


Figure 21

There are also examples of "molecular tweezer" receptors, where two host species, commonly crown ethers , are joined together via a suitable flexible or rigid bridge resulting in a molecular sandwich type of complex on inclusion of a suitable guest[76-79]. An example of such a compound and its metal complex crystal structure is shown in **Figure 22**[80].

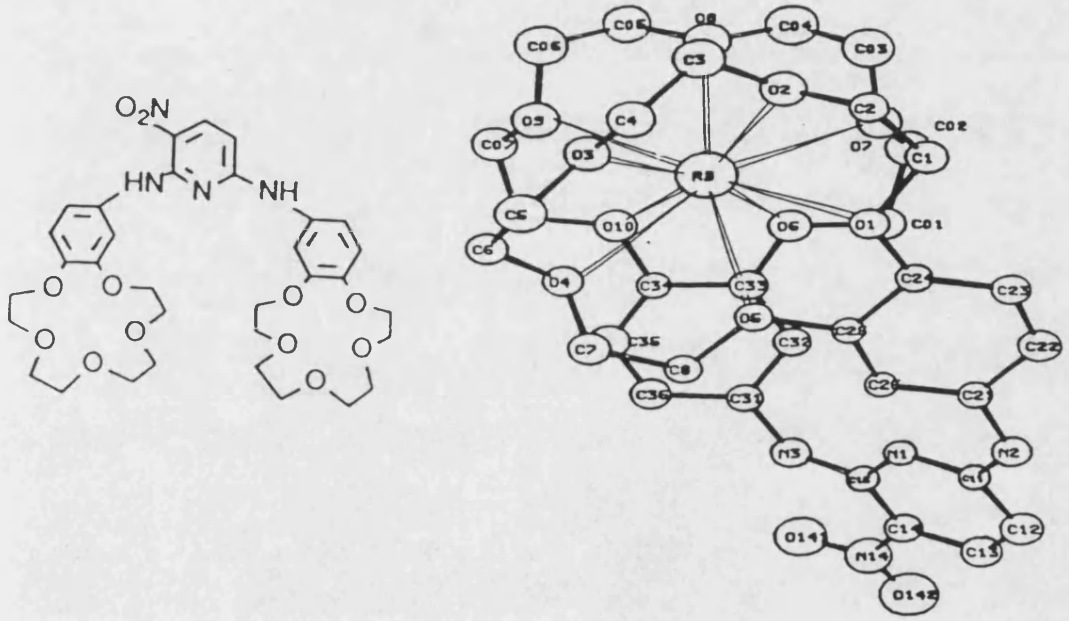


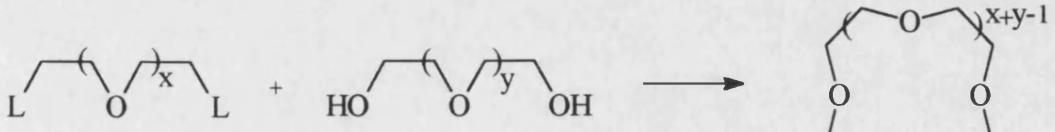
Figure 22

The rubidium(I) cation diameter (2.94Å) is larger than the benzo-15-crown-5 hole size (1.7-2.2Å) thus allowing the Rb^+ to form a stable intramolecular sandwich complex by linking the two adjacent crown ether rings. The ten Rb---O bond lengths average 2.93Å. The crown ether rings are almost parallel to each other, on either side of the cation providing a symmetrical co-ordination sphere. Despite being good donor atoms none of the nitrogen centres, from the pyridine and the two secondary amines, take part in any intramolecular bonding. Water and methanol arising from the solvent are also involved in the stabilisation of the solid-state structure.

1.3. Experimental Review

1.3.1. Preparation of Crown Ethers

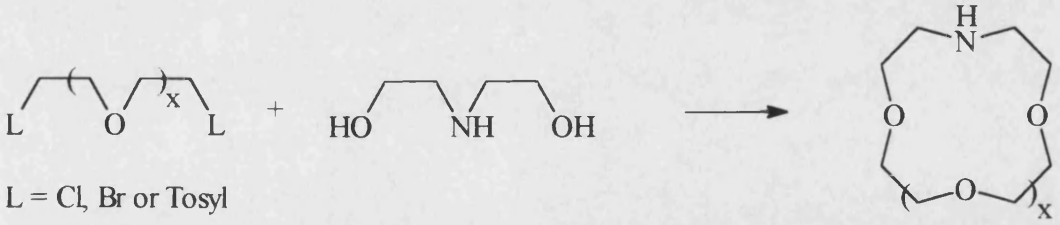
Since crown ethers were chosen as the basis for the receptors used in this investigation, their synthesis, and that of their nitrogen containing analogues, will be reviewed. As mentioned earlier the preparation of crown ethers relies on the formation of a cyclic product from two acyclic precursors (**Scheme 1**).



L = Cl, Br or tosyl

Scheme 1

From the above scheme it can be seen that the ring size is determined by the number of ether units in either of the starting materials. The preparation of simple aza-crown ethers is slightly different in that the ring size is more usually determined by the oligoethylene glycol (**Scheme 2**).



L = Cl, Br or Tosyl

Scheme 2

Since their first discovery, by a route which gave very low yields, there has been intense interest in improving the preparative methods for both oxa- and aza-crown ethers[81-94] and understanding the factors which maximise yields.

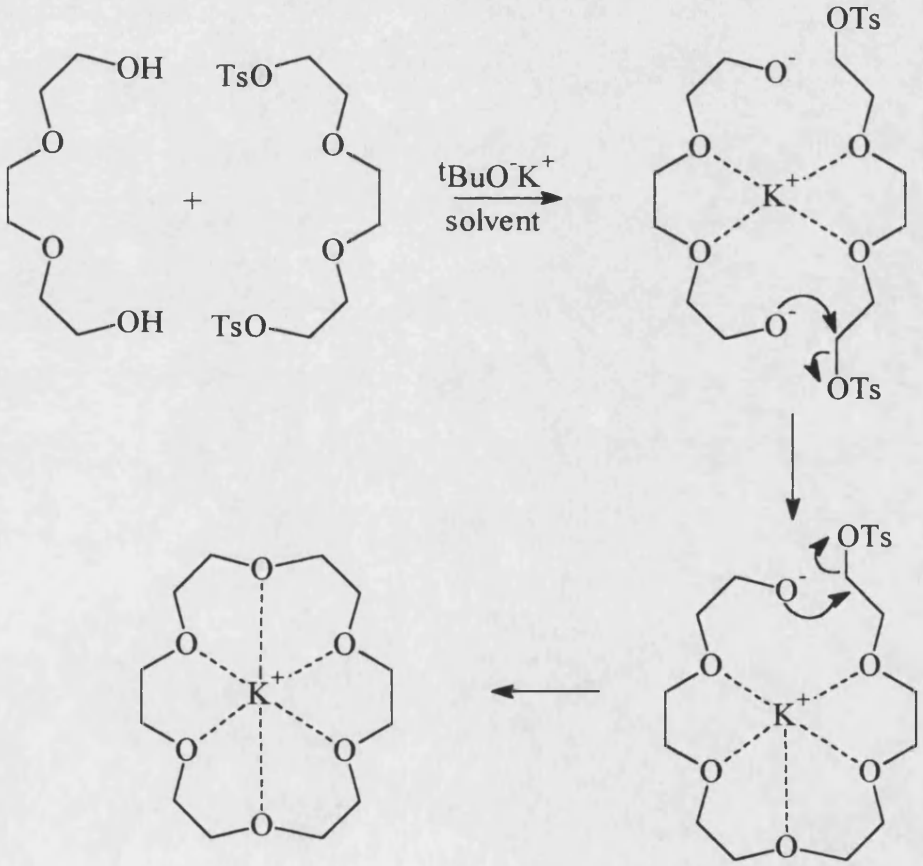
1.3.2. The Template Effect

The synthesis of macrocycles initially proved very time-consuming because of low yields, the formation of many, usually polymeric, side-products and the high dilution factors required to minimise polymerisation[95]. The ability of the co-ordination sphere of a metal ion to hold the reacting groups in the correct positions for cyclisation was not generally recognised until Busch and Thompson synthesised macrocycles using nickel(II) ions as templating agents[96-97].

There are three types of template effect. The first is the kinetic template effect which is defined as providing a route to the product, via the co-ordinating action of the metal on the reactants, which would not be available in the absence of the metal cation[98] The second type is the thermodynamic template effect. Macrocycles are formed in the absence of the metal ion, but macrocycle formation is promoted by the metal removing the product from the reaction equilibrium as a macrocycle metal complex[99]. Thirdly, there is the equilibrium template effect. In this case the reactants take part in a reversible reaction to give an intermediate which forms a stable complex with the metal. All the reactants then proceed to a macrocycle-metal complex. An important difference between the equilibrium and thermodynamic template effects is that in reactions involving the former different products are obtained for the metal-assisted and the metal-free reactions, but in reactions involving the latter, the same products are produced by the two reactions[100].

1.3.3. The Template Effect and Crown Ether Synthesis

Crown ethers readily incorporate an appropriately sized cation within their cavity, for example K^+ in 18C6, and this tendency has been used to promote the formation of a cyclic product at the expense of linear side-products. The cation acts as a stabilising centre around which the cyclisation occurs. The intermediate wraps around the cation, through ion-dipole interactions, which encourages ring closure by the final active end groups. The mechanism proposed by Greene[101] for the template effect is shown below (**Scheme 3**).

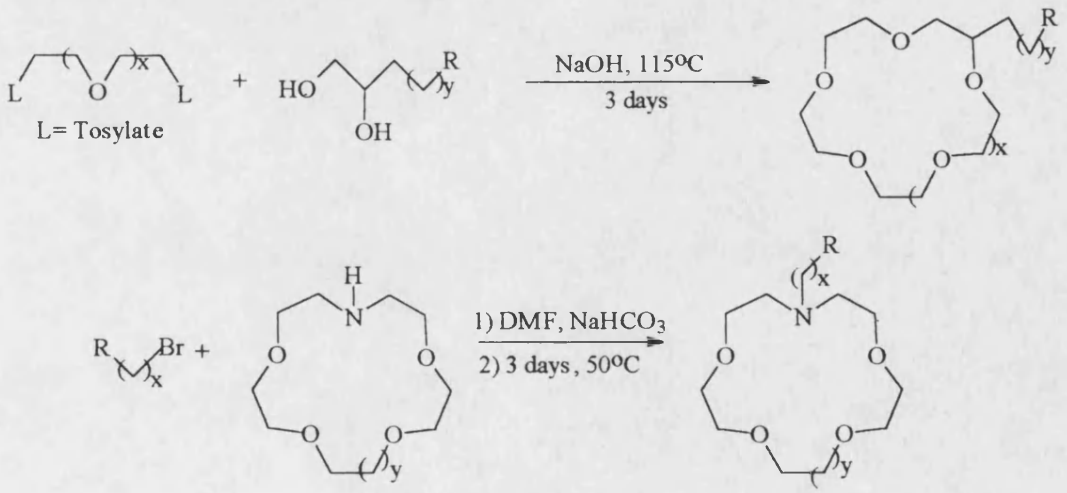


Scheme 3

The template effect is important in crown ether synthesis because the formation of a macrocycle is not favoured kinetically. However the necessity for introducing a particular sized metal cation, as opposed to any other metal cation is not proven[102]. Investigations by Reinhoudt et al[103] have shown that a variety of different metal fluorides can be used successfully in the synthesis of crown ethers. The fluorides of caesium and rubidium gave higher yields of crown ethers than the potassium salt, even in the synthesis of dibenzo-18-crown-6 in which the reverse would be expected. Tetrabutylammonium fluoride was ineffective. Thus the effectiveness of templating species may be determined by the environment in which they are to be employed, such as the solvent and the presence of reactive groups on the organic substrates, as well as the size / charge ratio of the templating ion.

1.3.4. Preparation of Functionalised Crown Ethers.

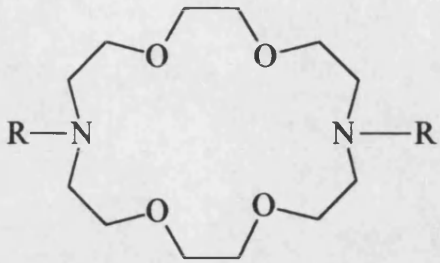
As indicated above, the selectivity and complexing ability of crown ethers can be modified by the introduction of side-arms, lariats, onto the ring, or by the joining of two or more crown ethers by either rigid or flexible bridge[104-113]. Examples of the former are illustrated in **Scheme 4**.



Where R = functional group (OH, OMe, ester etc.)

Scheme 4

The increased binding capacity, as shown by log K_f, of diaza-18-crown-6 functionalised with $-CH_3$ or CH_2CH_2OH, compared to that of unfunctionalised diaza-18-crown-6 is shown in **Table 1.5**.

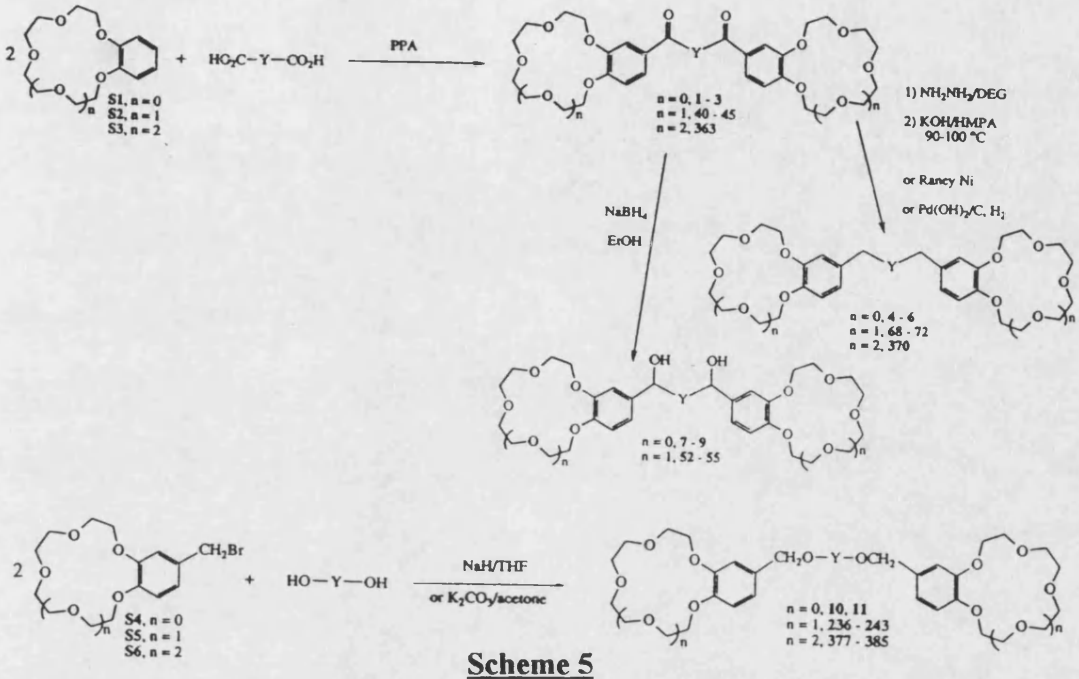


	Log K_f					
Substituent, R	Na^+	K^+	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
$-CH_3$	≤1	≤1	1.20	2.13	1.84	1.75
$-CH_2CH_2OH$	1.0	≤1	1.24	4.06	3.79	3.23

Table 1.5 The Additional Formation Constant for Difunctionalised Compared to Unfunctionalised Diaza-18-Crown-6 With Various Metal Cations (Measured in Water at 20°C).

Receptors based on two linked oxygen macrocycles frequently utilise an arene ring present as part of the macrocycle to provide a means of generating a bridge. This avoids the difficulty of introducing a bridge directly onto a sp^3 carbon atom in a methylene group within the crown ether. These bis(crown ethers) have found uses as ionophores in ion-selective electrodes since the co-operative action of the two adjacent crown ether units leads to a stronger complexation of specific metal ions than the corresponding mono-crown ether.

The most common bis(benzo crown ethers) use benzo-15-crown-5 as the receptor species because of its' relative cheapness and ready availability. The various methodologies in use have been reviewed by An et al[114], and some examples are shown in **Scheme 5**.



Scheme 5

1.3.5. Polymer Attachment

The attachment of crown ethers onto solid surfaces or polymeric species, in order to render them insoluble in aqueous media so making them useful in separation procedures, can be achieved by several methods. They may be attached to a carbon or silicon based polymer resulting in materials which may then be useful as liquid membranes[115], or bonded to a reactive porous surface such as silica gel or borosilicate to give a solid supported membrane[116-117] or a chromatographic stationary phase[118]. Various polymer supported crown ethers for use in liquid and gas chromatography have been synthesised from crown ethers containing a hydroxyl or an alkenyl group which permits their attachment to polystyrene[119], silica[120] or polysiloxane[121-122] backbones.

Alkenyl functionalised macrocyclic receptors may also be polymerised resulting in a range of organic-inorganic (e.g. polyphosphazenes, polysilanes)[123], "exotic organic" (polyacetylene, polyanaline)[124], and petrochemical polymers (polyolefins, polyethers)[125-127], as defined by Allcock[128]. In this study the mode of attachment has centred on hydrosilylation in which a hydride containing polysiloxane copolymer is linked to an active host via a terminal alkene functionality.

1.4. Membranes

Membranes are materials which are capable of mediating the passage of molecules or ions, often from one phase to another. There are many different types of membrane ranging from the naturally occurring biological membranes through to synthetic membranes which have found wide-spread use in industrial applications. Membranes can be divided into two groups, those with pores and those without . Porous membranes operate by a sieving mechanism. Membranes without pores, such as liquid membranes, frequently consist of an organic solvent in which is dissolved carrier molecules which reversibly complex substrates and assist in their transportation under the influence of an appropriate driving force[129-132] (**Figure 23**). Such membranes are of some relevance to this study.

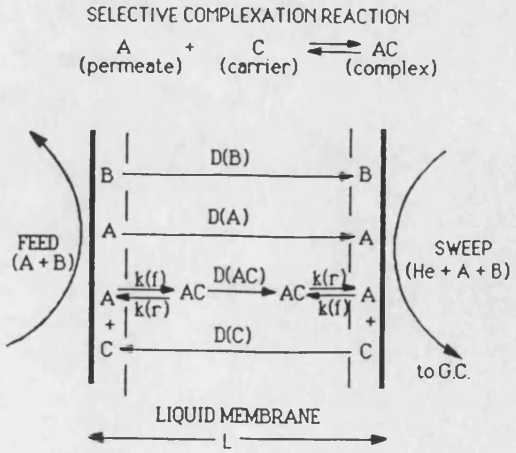


Figure 23

Liquid membranes containing carriers can be made highly selective by careful choice of the carrier and these carriers facilitate the transport of the substrate through the membrane from the feed to the sweep, as illustrated above. Carrier facilitated transport increases as the substrate concentration decreases through decreased competition for the transport sites.

These membranes have a number of disadvantages, particularly if they are used in conjunction with a porous solid resulting in an immobilised liquid membrane (ILM), in that they can be susceptible to solvent loss due to evaporation or displacement processes forcing the liquid out of the support pores. The complexing agent may also be lost from the membrane through partitioning between the bulk phase and the solvent, or by the membrane becoming deactivated via irreversible side-reactions[133].

Crown ethers and other host compounds have been attached as carrier sites to mobile, fluid supports giving solventless, integrated liquid membranes[134-135]. These membranes then become a supported liquid membrane (SLM) when sandwiched between a solid porous material which does not take an active part in transport process but enables the membranes to facilitate transport as a thin layer[136]. One of the commercially available synthetic supports is Celgard, a thin, opaque, microporous polypropylene flat sheet membrane, which has found wide use in these types of membranes[137]. Although SLMs have proved to be useful materials, they can require specialised apparatus in order to be used effectively[138].

1.4.1. Polyorganosiloxanes as Fluid Supports

Polysiloxanes are materials of commercial importance, with uses amongst others being implant materials in surgery and as hydrophobic coatings for glass, onto which organic groups can be immobilised[139-140] for specific purposes. Linear poly(dimethylsiloxane) itself has a number of intrinsic properties which can be utilised for various applications[141-142], but of particular importance in this study are its thermal and chemical stability, its low viscosity, and the ease with which the basic structure can be functionalised. Polyorganosiloxanes contain a backbone chain of Si-O linkages which leads to a highly flexible macromolecule since the Si-O-Si bond angle may vary between wide limits with little loss in Si-O bond strength.

The calculated potential energy versus the Si-O-Si bond angle, as determined by three computational approaches is shown in **Figure 24**[143]. Despite this flexibility the Si-O bond is formed through a favourable σ-bond resulting in a strong linkage with a bond enthalpy of approximately 370 kJ mol^{-1}, which confers high thermal stability to polysiloxanes. In addition the organic groups shield the polar Si-O units from chemical attack, and render the materials hydrophobic.

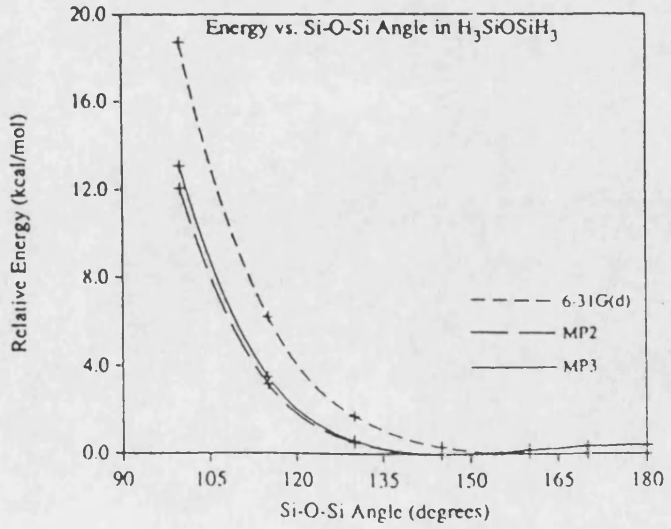
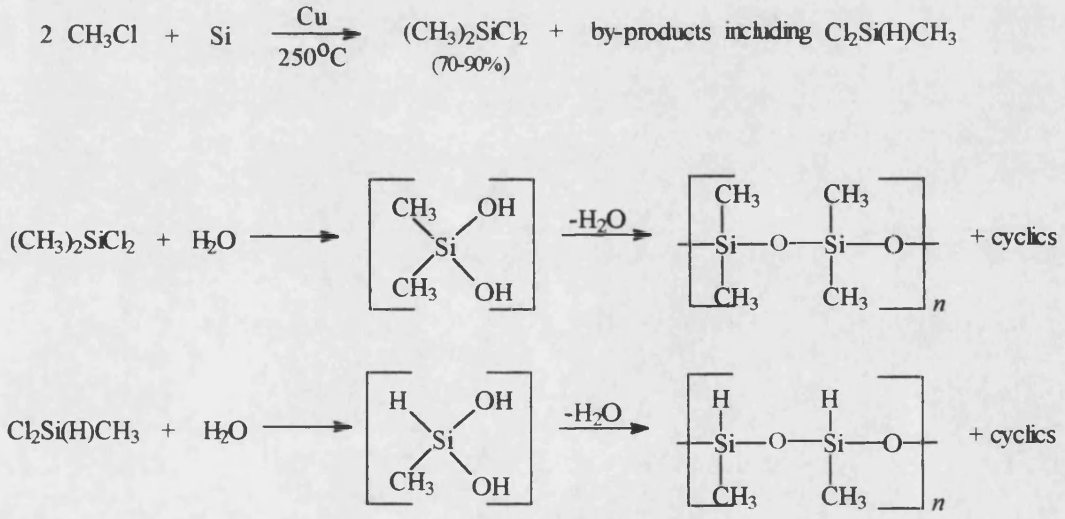


Figure 24

Functionalisation occurs most easily through modification of Si-H bonds and although the Si-H bond has approximately the same strength as a Si-O linkage it is susceptible to hydrolysis at elevated temperatures due to the polarising effect of the silicon ($Si^{\delta+}$-$H^{\delta-}$). Therefore moisture must be excluded during reactions at high temperature otherwise the hydride moiety will be hydrolysed to a silanol, followed by cross-linking on elimination of water from the silanol groups.

Polyorganosiloxanes are readily available from simple laboratory preparations or from commercial sources[144-145]. A simple general preparative route for polyorganosiloxanes is shown in **Scheme 6.**



Scheme 6

1.4.2 Organofunctional Polysiloxanes

Linear polymers with end- or side-chain Si-H groups permit the ready attachment of carrier sites onto the polymer backbone[146-147]. The degree and type of functionality gives the polymer specific properties for use in specific applications[113]. Although there are a number of reactions which can be utilised to attach functional groups onto a silicon centre, only the high yielding hydrosilylation reaction between a Si-H hydride functionality on the polymer and an alkene functional group on a suitably modified receptor will be considered further, as it is the method employed in this study.

Hydrosilylation reactions can usually be achieved by one of the following methods :-

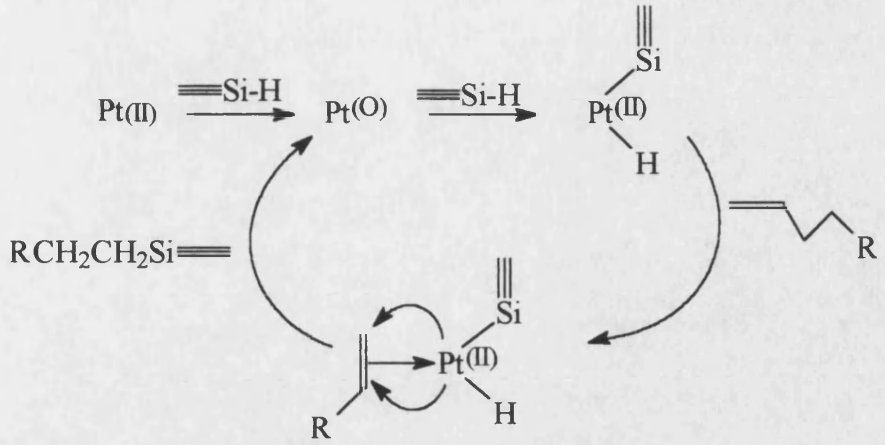
(i) by heating the reactants together at high temperature and pressure in the absence of a catalyst.

(ii) by adding an organic peroxide or an azo-compound as a radical initiator.

(iii) by exposing the reactants to either uv- or γ- radiation, or to an electrical discharge.

(iv) by adding a catalyst, such as a transition-metal salt or a Lewis base (e.g. pyridine) to the reaction mixture.

The use of metal species from groups 9 and 10, such as rhodium(I) and platinum(II) derivatives have been widely reported for use as homogeneous catalysts[148-149]. A basic mechanism for the hydrosilylation was proposed by Chu and Frye[150]. It involves the oxidative-addition and reductive-elimination of the organic species on the metal centre (**Scheme 7**).



Scheme 7

Hydrosilylation reactions using methods (ii) and (iii) offer the most practical alternative routes should the transition-metal salt route prove ineffective, since they can be carried out at low temperatures, thus avoiding the possibility of cross-linking.

1.4.3. The Use of Crown Ethers in Liquid Membranes.

The use of macrocycles as polymer attached carriers in supported liquid membranes has been widely documented and is the subject of a review by van Straaten-Nijenhuis et al[151]. The first macrocycle mediated cation transport through a supported liquid membrane was reported by Reusch and Cussler[152] who studied the selective transport of various metal cations across a dibenzo-18-crown-6 containing membrane. They noted the selective transport of K^+ and Pb^{2+} compared to other mono- and divalent cations respectively.

The influence of the macrocyclic ligand structure on carrier-facilitated cation transport rates and selectivities through liquid membranes has been studied by Lamb et al[153]. They determined the rate of transport of the nitrate salts of Li^+, Na^+, K^+, Cs^+, Ag^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and Pb^{2+} through stirred bulk membranes using a large number of macrocycle ligand carriers. The macrocycles were crown ethers and cryptands with the maximum ring size being equivalent to that of 21-crown-7.

Transport selectivity was observed for those cations which best fitted the ligand cavity as long as the complex stability constant, K_f, was not so large as to permanently bind the guest cation. The effects of the macrocycle structural features were also determined, with benzo-substituted analogues of crown ethers giving lower transport rates than cyclohexano substituted or unsubstituted crown ethers. The addition of large aliphatic groups which served to minimise loss of the carrier to the aqueous phase did not significantly alter transport rates or selectivities.

The applications of macrocycles functionalised with lariats which would offer additional cation-binding sites through hydrogen bonding, particularly carboxylic acid side chains have been studied by Bartsch et al[154-155] and Fyles et al[156-157]. Their studies were based primarily on 2-(sym-dibenzo-16-crown-5-oxy)-decanoic acid and 2R,3R,11R,12R-3,11-Bis[(octylamino)carbonyl]-1,4,7,10,13,16-hexaoxacyclooctadecane-2,12-dicarbonyl respectively. The position on the macrocycle with respect to other substituents and the length of the spacer chain was found to be of particular importance. Superior transport selectivity was seen for Na^+ for a macrocycle whose orientation of the lipophilic groups on the acid side-chain forces the carboxylate group to lie directly over the centre of the polyether ring.

The transport of biologically important compounds, particularly amino-acids, has also been studied by an extension of the principles involved in metal cation transport. Bryjak et al[158-159] reported the mediated transport of the hydrochlorides of phenylalanine and aspartic acid, and also free 3-amino-3-phosphoproprionic acid and phenylalanine through an immobilised liquid membrane using Krypofix-5 as the carrier. Increasing the concentration of the carrier increased the rate of transport. The fluxes of all four compounds were essentially the same suggesting the absence of selective transport.

In a more sophisticated study carried out by Shinbo et al[160] the R-2,3,4,5-bis(1,2-(3-phenylnaphto))-1,6,9,12,15,18-hexaoxacycloeicosa-2,4-diene was used in the form of a supported liquid membrane for the mediated enantioselective transport of D/L phenylalanine, D/L tryptophan, and D/L isoleucine respectively from aqueous solution. The selective transport of the D-enantiomer of all three amino-acids was achieved. On using the S-chiral crown ether the L-enantiomer was preferentially transported.

1.5. Research Programme Using Siloxane Supported Crown Ethers for the Selective Complexation of Specific Organic Compounds

The main targets of the research programme can be summarised as follows :-

i) The identification of simple crown ethers which may be appropriate for use as receptors in host-guest interactions of targeted organic species containing amine and / or acid functionalities.

ii) The preparation and chemical characterisation of crown ethers containing a suitable side chain functionality which would allow attachment onto a polymeric fluid support.

iii) Studies of host-guest interactions between host and guest using n.m.r methods

iv) The attachment of functionalised receptors to model and short chain fluid siloxane polymers and characterisation of the resultant functionalised polymer.

v) The development of sensitive analytical methods for amino-acid quantification.

vi) The synthesis, characterisation and evaluation of more sophisticated host compounds based on more complex crown ether receptors, in an attempt to maximise interactions with the specific organic hosts.

vii) The evaluation of appropriately functionalised siloxane polymers as separants for the target organic species dissolved in aqueous solutions.

Chapter Two

Synthetic Techniques

2.1. Summary

A range of derivatised crown ethers has been synthesised, and their capacity to act as receptors for biorelevant molecules, particularly glutamic acid and GABA, has been studied. The receptors consist of mono-aza-15-crown-5, and mono- and diaza-18-crown-6 containing N-alkenyl functionalities, which permit subsequent attachment to a polysiloxane support via a hydrosilylation reaction.

Some of these alkenyl functionalised aza-crown ether analogues have themselves been subject to further modification, including oxidation of the alkene functionality in order to prepare lariat crown ethers and so improve their ability to bind specific guests. The synthesis of oxygen crown ether derivatives, and their subsequent immobilisation on a polymeric support is also described. The preparations of compounds containing two mono-aza-18-crown-6 moieties linked by a carbon backbone are then discussed.

The preparative techniques used to synthesise functionalised model- and poly-siloxanes are described before finally giving the details of miscellaneous reactions, including the preparation of intermediates and important syntheses which were attempted but which were not successful. All of the compounds isolated have been fully characterised using an appropriate range of analytical methods as described in Chapter 3.

2.2. Synthetic Procedures

2.2.1. Functionalised Crown Ethers

The mono-aza-15-crown-5 and -18-crown-6 precursors were prepared by the method described by Maeda et al[93], or by simple modifications thereof with yields of around 70%. Some were also obtained from commercial sources and used without

further purification. The preparation of functionalised mono-, di-aza- and oxygen crown ethers followed the general procedures reported previously[113]. N-alkenyl chains were introduced onto aza-crown ethers via a simple nucleophilic substitution reaction using an appropriate n-bromoalkene, so generating a range of N-alkenyl mono-aza-crown ethers with side-chains of 3,4,6, and 8 carbon atoms. A similar route was used for the introduction of -diol and carboxylic acid-containing side chains. The carbon-functionalised oxygen crown ethers, based upon either 12 or 15 membered macrocycles, were synthesised from an alkenyl-1,2-diol compound and an appropriate oligoethylene glycol di-p-tosylate. The pure products were usually isolated after solvent extraction followed by careful distillation under reduced pressure.

The syntheses of bis(mono-aza-18-crown-6) compounds were attempted employing the same route as that described above[112] using either a diacid or its acid chloride to form the bridging link between the crown centres. Syntheses using the diacid directly used the methods, or modifications thereof, of Neises et al[161] and Sheehan et al[162]. The resulting bis-crown ethers could not be distilled under reduced pressure as degradation occurred at temperatures above 125^{o}C. However, gentle distillation under reduced pressure below 100^{o}C removed unreacted starting materials, which otherwise were still present following column chromatography of the reaction products.

2.2.2. Crown Ether Functionalised Model Siloxanes and Polysiloxanes

Functionalised mono-aza-15-crown-5, mono-aza-18-crown-6 and oxygen 15-crown-5 were immobilised onto linear siloxanes via a hydrosilylation reaction between the alkenyl terminated crown ether and one or more Si-H moieties in the siloxane. This reaction was catalysed by a platinum salt[163], and it was carried out in

a sealed tube using the method of Abed-Ali et al[113]. In this way air and moisture was easily excluded, and in addition the reaction proceeded at slightly elevated pressure as well as temperature, with concomitant improvement in yields.

Initially commercially available trisiloxanes were used to assess the effectiveness of the catalyst in the hydrosilylation procedure. Once appropriate reaction conditions had been determined, reactions were extended to polymeric siloxanes containing known mole fractions of Si-H linkages. Both the commercial siloxane starting materials and the products derived from them were analysed by proton n.m.r. spectroscopy in order to confirm purity, and also to verify the degree of functionality before and after the hydrosilylation reaction.

2.3. Experimental

2.3.1. Introduction

The precursors used were either prepared in the course of this study, or obtained from commercial sources (Aldrich, Fluka, Lancaster and ABCR) and then used without further purification. Solvents were purified by standard methods and then stored over appropriate drying agents under a dry nitrogen atmosphere. Reactions involving siloxanes were routinely carried out in the complete absence of air and moisture.

2.3.2. Synthesis of Functionalised Crown Ethers

Synthesis of 1,4,7,10-tetraoxa-13-azacyclopentadecane (N15C5)

To a stirred solution of diethanolamine (6.3g, 60mmol) and sodium metal (5.0g, 210mmol) in ^{t}BuOH (400cm^3) held at 40^{o}C was added dropwise over 2 hours a solution of triethylene glycol-p-ditosylate (12.6g, 30mmol) in THF (100cm^3). The resulting solution was stirred for a further 2 hours at that temperature and then

filtered. The precipitate was washed with DCM ($100cm^3$). The filtrate and the washings were combined and the solvent was then removed *in vacuo*. Water ($20cm^3$) was added to the residue and the solution extracted first with hexane ($2\times 20cm^3$) and then with DCM ($5\times 20cm^3$). The DCM extractions were combined and dried over $MgSO_4$. The solution was then filtered and the solvent removed in vacuo. The residue was then distilled (150°C, 0.1mmHg) to give an oil which solidified on standing to give a white solid (3.6g, 55%) which had a melting point of 36-37°C.

I.R.; ν_{max}	3316 (N-H str) 2866 (C-H str) 1128 (C-O str)
δ_H ($CDCl_3$)	3.55 (16H, m, -C<u>H</u>$_2$-O-C<u>H</u>$_2$-) 2.85 (1H, br s, >N-<u>H</u>)
	2.69 (4H, m, -C<u>H</u>$_2$-NR-C<u>H</u>$_2$-)
δ_C ($CDCl_3$)	70.0, 69.7, 69.4 (-<u>C</u>H$_2$-O-<u>C</u>H$_2$-) 48.7 (-<u>C</u>H$_2$-NR-<u>C</u>H$_2$-)
m/e (E.I.)	219 $(M)^+$ 51%, 188 $(M\text{-}CH_3O)^+$ 18%, 162 $(M\text{-}C_3H_7N)^+$
	23%, 43 $(M\text{-}C_8H_{16}O_3)^+$ 100%.
Analysis; found :-	C, 54.1; H, 10.1; N, 6.36%. $C_{10}H_{21}NO_4$ requires :-
	C, 54.8; H, 9.59; N, 6.39%.

<u>Synthesis of 13-(1-propen-3-yl)-1,4,7,10-tetraoxa-13-azacyclopentadecane (PN15C5) (Route 1)</u>

Allyl bromide (1.3g, 11mmol) in DMF ($50cm^3$) was added dropwise to a stirred solution of N15C5 (2.0g, 9.1mmol) and $NaHCO_3$ (1.0g, 12mmol) in DMF ($30cm^3$). The mixture was heated to 50°C and stirred at that temperature for a further 3 days. The reaction mixture was then poured into water ($20cm^3$) and the solution extracted with DCM ($5\times 40cm^3$). The extractions were combined and dried over $MgSO_4$. After filtration solvent was removed *in vacuo*. The residue was distilled (160°C, 0.1mmHg) to give a clear, colourless oil (0.7g, 28%).

I.R.; ν_{max}	3076 (alkene C-H str) 2860 (C-H str) 1676 (C=C str)
	1126 (C-O str)
δ_H ($CDCl_3$)	5.85 (1H, m, =C<u>*H*</u>-) 5.05 (2H, m, =C<u>*H*$_2$</u>, J = 16.5 (trans),
	12.0 (cis), 0.8 Hz (gem)) 3.63 (16H, m, -C<u>*H*$_2$</u>-O-C<u>*H*$_2$</u>-)
	3.10 (2H, d, >N-C<u>*H*$_2$</u>-CH=, J = 6.0 Hz)
	2.76 (4H, t, -C<u>*H*$_2$</u>-NR-C<u>*H*$_2$</u>-, J = 6.0 Hz)
δ_C ($CDCl_3$)	135.7 (=<u>C</u>H-) 117.2 (=<u>C</u>H$_2$) 70.9, 70.8, 70.4, 70.2, 69.9,
	69.7 (-<u>C</u>H$_2$-O-<u>C</u>H$_2$-) 59.8 (-<u>C</u>H$_2$-CH=)
	53.9 (-<u>C</u>H$_2$-NR-<u>C</u>H$_2$-)
m/e (C.I.)	260 $(M+H)^+$ 16%, 187 $(M-C_4H_8O)^+$ 76%,
	146 $(M-C_7H_{13}NO)^+$ 42%
Analysis; found :-	C, 60.5; H, 10.2; N, 5.20%. $C_{13}H_{25}NO_4$ requires :-
	C, 60.2; H, 9.65; N, 5.41%.

<u>Synthesis of 13-(1-buten-4-yl)-1,4,7,10-tetraoxa-13-azacyclopentadecane (BN15C5).</u>

The preparation followed an analogous procedure to that given above but using 4-bromobut-1-ene (1.5g, 11mmol), N15C5 (2.0g, 9mmol) and $NaHCO_3$ (1.0g, 12mmol) in DMF (50cm^3). The final residue was distilled (160^oC, 0.1mmHg) to give a clear, colourless oil (2.8g, 57%).

I.R.; ν_{max}	3074 (alkene C-H str) 2937, 2860 (C-H str) 1639 (C=C str)
	1130 (C-O str)
δ_H ($CDCl_3$)	5.78 (1H, oct, =C<u>*H*</u>-) 5.00 (2H, m, =C<u>*H*$_2$</u>, J = 18.0 (trans),
	10.5 (cis), 1.5 Hz (gem)) 3.63 (16H, m, -C<u>*H*$_2$</u>-O-C<u>*H*$_2$</u>-)
	2.78 (4H, t, -C<u>*H*$_2$</u>-NR-C<u>*H*$_2$</u>-, J = 6.0 Hz)
	2.58 (2H, t, >N-C*H*$_2$-, J = 7.5 Hz)
	2.20 (2H, q, -C<u>*H*$_2$</u>-CH=, J = 7.5 Hz)

δ_C ($CDCl_3$) 136.5 (=$\underline{C}$H-) 115.3 (=$\underline{C}H_2$) 70.2, 70.1, 70.0, 69.9, 69.6 (-$\underline{C}H_2$-O-$\underline{C}H_2$-) 54.3 (>N-$\underline{C}H_2$-) 31.6 (-$\underline{C}H_2$-CH=)

m/e (C.I.) 274 $(M+H)^+$ 98%, 232 $(M-C_3H_7)^+$ 100%.

Analysis; found :- C, 61.4; H, 10.1; N, 5.08%. $C_{14}H_{27}NO_4$ requires :- C, 61.5; H, 9.89; N, 5.13%.

Synthesis of 13-(hexen-6-yl)-1,4,7,10-tetraoxa-13-azacyclopentadecane (HN15C5)

This compound was prepared as above but using 6-bromohex-1-ene (1.5g, 9.1mmol), N15C5 (2.0g, 9.1mmol) and $NaHCO_3$ (1.0g, 12mmol) in DMF (50cm^3). It was isolated as a clear, pale-yellow oil (2.0g, 74%).

I.R.;ν_{max} 3074 (alkene C-H str) 2934, 2860 (C-H str) 1639 (C=C str) 1352 (C-H bend) 1130 (C-O str)

δ_H ($CDCl_3$) 5.80 (1H, oct, =C$\underline{H}$-) 4.95 (2H, m, =C$\underline{H}_2$, J = 18.0 (trans), 10.5 (cis), 0.8 Hz (gem)) 3.65 (16H, m, -C$\underline{H}_2$-O-C$\underline{H}_2$-) 2.75 (4H, t, -C$\underline{H}_2$-NR-C$\underline{H}_2$, J = 6.0 Hz) 2.50 (2H, t, >N-C$\underline{H}_2$-, J = 7.5 Hz) 2.05 (2H, q, -C$\underline{H}_2$-CH=, J = 7.5 Hz) 1.40 (4H, m, -C$\underline{H}_2$-C$\underline{H}_2$-CH_2CH=).

δ_H ($CDCl_3$) 139.3 (=$\underline{C}$H-) 114.9 (=$\underline{C}H_2$) 70.9, 70.8, 70.6, 70.5, 70.4, 70.3 (-$\underline{C}H_2$-O-$\underline{C}H_2$-) 57.3, 55.1 (-$\underline{C}H_2$-NR-$\underline{C}H_2$-) 49.6 (>N-$\underline{C}H_2$-) 34.1 (-$\underline{C}H_2$-CH=) 27.3, 27.2 (=CHCH_2-$\underline{C}H_2$-)

m/e (E.I.) 301 $(M)^+$ 12%, 232 $(M-C_5H_9)^+$ 100%, 218 $(M-C_6H_{11})^+$ 5%.

Analysis; found :- C, 63.1; H, 10.6; N, 4.85%. $C_{16}H_{31}NO_4$ requires :- C, 63.8; H, 10.3; N, 4.65%.

Attempted synthesis of 13-allyl-1,4,7,10-tetraoxa-13-azacyclopentadecane (PN15C5) (Route 2)

Di-(2-hydroxyethyl)allylamine (6.0g, 40mmol), and sodium metal (2.1g, 90mmol) were dissolved under nitrogen in ^{t}BuOH (250 cm^3) and the mixture was heated to 60°C. Triethylene glycol dichloride (7.5g, 40mmol) in dioxan (150cm^3) was then added dropwise to the solution over a three hour period. The reaction was continued for a further 40 hours after which time the mixture was filtered and the solvent was evaporated from the filtrate. Water (25cm^3) was added to the residue and the solution was extracted once with hexane (20cm^3) and then extracted with DCM (5×20cm^3). The combined DCM extracts were dried using $MgSO_4$ and solvent removed *in vacuo* from the filtered solution. The crude oil was distilled (125°C, 0.1mmHg) to give a clear, pale-yellow oil (4.8g, 47%).

I.R.; ν_{max}	3317 (O-H str) 2866 (C-H str) 1674 (C=C str) 1124 (C-O str)
δ_H ($CDCl_3$)	5.85 (1H, m, =C*H*-) 5.16 (2H, m, CH_2=, J =12.0 (trans), 9.0 (cis), 1.5 Hz (gem)) 3.6 (16H, m, -CH_2-O-) 3.05 (2H, m, >N-CH_2-CH=) 2.8 (2H, m, >N-CH_2-CH_2-O-)
δ_C	134.7 (=CH-) 117.8 (=CH_2) 71.2, 70.7, 70.4, 70.1 (-CH_2-O-CH_2-) 59.7 (-CH_2-CH=) 53.9 (-CH_2-NR-CH_2-)
m/e (E.I.)	259 (M^+); (C.I.); 260 $(M+H)^+$

Proton n.m.r spectroscopy indicated the presence of unreacted starting materials and also side-products, so no CHN analytical data were obtained.

Synthesis of 1,4,7,10,13-pentaoxa-16-azacyclooctadecane (N18C6).

Under a nitrogen atmosphere diethanolamine (8.1g, 80mmol) and potassium metal (CARE) (3.9 g, 100mmol) were dissolved in ^{t}BuOH (300cm^3), and a solution of tetraethylene glycol-p-ditosylate (19.2g, 38mmol) in THF (100cm^3) was added dropwise over a 2 hour period to the stirred solution held at 40^{o}C. After the addition was complete the reaction was continued for a further 2 hours. The mixture was then filtered, the precipitate washed with DCM (100cm^3), and the filtrate combined with these washings. Solvent was then removed *in vacuo*. Water (20cm^3) was added to the residue, and the solution was extracted twice with hexane (20cm^3) and then with DCM (5×20cm^3). The DCM extracts were combined and the solvent was removed *in vacuo*. The residue was distilled (150^{o}C, 0.1mmHg) yielding the required product as a white solid (7.1g, 70%) with a melting point of 45-46^{o}C.

I.R.; ν_{max}	3196 (N-H str) 2951, 2878, 2814 (C-H str) 1120 (C-O str)
δ_H ($CDCl_3$)	3.65 (20H, m, -C$\underline{H}_2$-O-C$\underline{H}_2$-) 2.79 (4H, t, -C$\underline{H}_2$-NR-C$\underline{H}_2$-, J = 4.5 Hz) 2.0 (1H, br s >N$\underline{H}$)
δ_C ($CDCl_3$)	71.5, 70.3 (-$\underline{C}$H$_2$-O-$\underline{C}$H$_2$-) 49.6 (-$\underline{C}$H$_2$-NR-$\underline{C}$H$_2$-)
m/e (E.I.)	263 $(M+H)^+$ 2%, 232 $(M-CH_2O)^+$ 27%, 74 $(M-C_9H_{18}NO_3)^+$ 100%.
Analysis; found :-	C, 53.4; H, 9.60; N, 5.40%. $C_{12}H_{25}NO_5$ requires :- C, 54.8; H, 9.51; N, 5.32%.

Synthesis of 16-(1-propen-3-yl)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (PN18C6)

A stirred mixture of N18C6 (2.0 g, 7.5mmol) and $NaHCO_3$ (0.7g, 8.3mmol) in DMF (40cm^3) was treated dropwise at room temperature with allyl bromide (0.7cm^3, 8.2mmol) in DMF (10cm^3). The mixture was then heated to 50^{o}C and

stirred at that temperature for a further 3 days. The mixture was then poured into water ($20cm^3$) and extracted with DCM ($5\times20cm^3$). The combined extracts were first dried over $MgSO_4$ and then solvent was removed *in vacuo*. The residue was finally distilled (200°C, 0.07mmHg) to give a clear, orange oil (0.9g, 39%).

I.R.; ν_{max}	3074 (C=C str) 2866 (C-H str) 1124 (C-O str)
δ_H ($CDCl_3$)	5.80 (1H, m, =C*H*-) 5.15 (2H, m, =C*H*$_2$, J = 15.0 (trans), 9.0 (cis), 1.5 Hz (gem)) 3.65 (20H, m, -C*H*$_2$-O-C*H*$_2$-) 3.17 (2H, d, >N-C*H*$_2$-, J = 5.0 Hz) 2.77 (4H, t, -C*H*$_2$-NR-C*H*$_2$-, J = 6.0 Hz)
δ_C ($CDCl_3$)	135.5 (=CH-) 117.2 (=CH_2) 70.6, 70.4, 70.1, 69.4 (-CH_2-O-CH_2-) 58.7 (-CH_2-NR-CH_2-) 53.3 (>N-CH_2-)
m/e (E.I.)	303 $(M)^+$ 15%, 98 $(M-C_{10}H_{21}O_4)^+$ 100%.
Analysis; found :-	C, 58.7; H, 9.80; N, 4.50%. $C_{15}H_{29}NO_5$ requires :- C, 59.4; H, 9.57; N, 4.62%.

Synthesis of 16-(1-buten-4-yl)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (BN18C6)

This crown ether was prepared by a similar procedure to that given above, using 4-bromobut-1-ene (1.1g, 8.2mmol), N18C6 (2.0g, 7.6mmol) and $NaHCO_3$ (0.8g, 9.5mmol) in DMF ($50cm^3$). The residue was distilled (160°C, 0.1mmHg) to give the product as a clear, colourless oil (1.2g, 49%).

I.R.; ν_{max}	3074 (alkene C-H str) 2937, 2860 (C-H str) 1639 (C=C str) 1354 (C-H bend) 1128 (C-O str)
δ_H ($CDCl_3$)	5.80 (1H, oct, =C*H*-) 5.05 (2H, m, =C*H*$_2$, J = 18.0 (trans), 10.5 (cis), 0.7 Hz (gem)) 3.65 (20H, m, -C*H*$_2$-O-C*H*$_2$-)

2.77 (4H, t, -C$\underline{H}_2$-NR-C$\underline{H}_2$-, J = 6.0 Hz)

2.60 (2H, t, >N-C$\underline{H}_2$-, J = 6.0 Hz)

2.10 (2H, q, -C$\underline{H}_2$-CH=, J = 6.0 Hz)

δ_C ($CDCl_3$) 136.6 (=$\underline{C}$H-) 115.2 (=$\underline{C}H_2$) 70.6, 70.5, 70.2, 69.7 (-$\underline{C}H_2$-O-$\underline{C}H_2$-) 55.2 (-$\underline{C}H_2$-NR-$\underline{C}H_2$-) 53.7 (>N-$\underline{C}H_2$-)

31.5 (-$\underline{C}H_2$-CH=)

m/e (C.I.) 318 $(M+H)^+$ 100%, 276 $(M-C_3H_7)^+$ 80%.

Analysis; found :- C, 60.7; H, 4.51; N, 10.2%. $C_{18}H_{31}NO_5$ requires :- C, 60.6; H, 4.42; N, 9.78%.

Synthesis of 16-(1-hexen-6-yl)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (HN18C6).

A similar procedure to that given above, using 6-bromohex-1-ene (3.0cm^3, 21mmol), N18C6 (5.0g, 23mmol) and $NaHCO_3$ (2.0g, 23mmol) in DMF (50cm^3), yielded the product after a final distillation (150°C, 0.1mmHg) as a clear, colourless oil (4.4g, 66%).

I.R.; ν_{max} 3074 (alkene C-H str) 2932, 2862 (C-H str) 1639 (C=C str) 1352 (C-H bend) 1124 (C-O str)

δ_H ($CDCl_3$) 5.80 (1H, oct, =C$\underline{H}$-) 5.00 (2H, m, =C$\underline{H}_2$, J = 18.0 (trans), 9.8 (cis), 2.3 Hz (gem)) 3.63 (20H, m, -C$\underline{H}_2$-O-C$\underline{H}_2$-)

2.75 (4H, t, -C$\underline{H}_2$-NR-C$\underline{H}_2$-, J = 6.0 Hz)

2.50 (2H, t, >N-C$\underline{H}_2$-, J = 6.8 Hz)

2.05 (2H, q, -C$\underline{H}_2$-CH=, J = 6.0 Hz)

1.40 (4H, m, >NCH_2-C$\underline{H}_2$-C$\underline{H}_2$-)

δ_C ($CDCl_3$)	138.7 (=<u>C</u>H-) 114.2 (=<u>C</u>H_2) 70.7, 70.6, 70.2, 69.8 (-<u>C</u>H_2-O-<u>C</u>H_2-) 55.7 (>N-<u>C</u>H_2-) 53.8 (>NCH_2-<u>C</u>H_2-) 33.5 (-<u>C</u>H_2-CH=) 26.5 (-<u>C</u>H_2-CH_2CH=)
m/e (C.I.)	345 $(M)^+$ 8%, 276 $(M-C_5H_{11})^+$ 100%
Analysis; found :-	C, 61.5; H, 10.2; N, 4.05%. $C_{18}H_{35}NO_5$ requires :- C, 62.6; H, 10.2; N, 4.06%.

<u>Synthesis of 16-(1-hexan-6-ol)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (HOLN18C6).</u>

The compound was synthesised as above from 6-bromohexan-1-ol (2.7cm^3, 15mmol), N18C6 (3.0g, 11mmol) and $NaHCO_3$ (1.3g, 15mmol) in DMF (50cm^3). After work-up the residue was distilled (190°C, 0.06mmHg) to give a clear, colourless oil (2.0g, 53%).

I.R.; ν_{max}	3362 (O-H str) 2930, 2860 (C-H str) 1120 (C-O str)
δ_H ($CDCl_3$)	3.65 (22H, m, -C<u>H</u>$_2$-O-C<u>H</u>$_2$, -C<u>H</u>$_2$-OH) 2.78 (4H, m, -C<u>H</u>$_2$-NR-C<u>H</u>$_2$-) 2.54 (2H, m, R-C<u>H</u>$_2$-NR_2) 1.40 (8H, m, HO-CH_2-(C<u>H</u>$_2$)$_4$-CH_2NR_2)
δ_C ($CDCl_3$)	70.6, 70.5, 70.2, 69.7 (-<u>C</u>H_2-O-<u>C</u>H_2-) 62.3 (-<u>C</u>H_2-OH) 55.7 (>N-<u>C</u>H_2-) 53.8 (-<u>C</u>H_2-NR-<u>C</u>H_2-) 32.5 (-<u>C</u>H_2-CH_2N<) 27.0 (-<u>C</u>H_2-CH_2CH_2N<) 25.4 (-<u>C</u>H_2-CH_2CH_2OH)
m/e (C.I.)	363 $(M)^+$ 54%, 291 $(M-C_4H_8O)^+$ 36%, 277 $(M-C_5H_{10}O)^+$, 263 $(M-C_6H_{12}NO_5)^+$ 100%
Analysis; found :-	C, 59.4; H, 10.5; N, 3.86%. $C_{18}H_{37}NO_6$ requires :- C, 59.5; H, 10.2; N, 3.86%.

Synthesis of 16-(1-hexan-6-oic acid)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (HOICN18C6)

A similar procedure to that described above using 6-bromohexan-1-oic acid (0.9g, 4.4mmol), N18C6 (1.0g, 3.8mmol) and $NaHCO_3$ (0.4g, 5.0mmol) in DMF ($50cm^3$) yielded the product after distillation (200°C, 0.05mmHg) as a clear, colourless oil (4..4g, 66%).

I.R.; ν_{max}	3530 (O-H str) 2872 (C-H str) 1674 (C=O str)
	1389 (C-H bend) 1113 (C-O str)
δ_H ($CDCl_3$)	3.65 (20H, m, -C<u>H</u>$_2$-O-C<u>H</u>$_2$-) 2.82 (4H, t, -C<u>H</u>$_2$-NR-C<u>H</u>$_2$-,
	J = 6.0 Hz) 2.40 (2H, p, >N-C<u>H</u>$_2$-, J = 5.0 Hz)
	1.63 (4H, m, >NCH_2-C<u>H</u>$_2$-, -C<u>H</u>$_2$-CH_2CO_2H)
	1.43 (2H, m, >NCH_2CH_2-C<u>H</u>$_2$-)
δ_C ($CDCl_3$)	173.0 (-<u>C</u>O_2H) 70.3, 70.1, 70.0, 69.7 (-<u>C</u>H_2-O-<u>C</u>H_2-) 62.6
	(-<u>C</u>H_2-NR-<u>C</u>H_2-) 48.9 (>N-<u>C</u>H_2-) 34.2 (-<u>C</u>H_2-CO_2H-)
	127.8 (>NCH_2-<u>C</u>H_2-) 24.3 (>NCH_2CH_2-<u>C</u>H_2-)
	24.2 (-<u>C</u>H_2-CH_2CO_2H)
m/e (C.I.)	377 $(M)^+$ 15%, 305 $(M-C_3H_4O_2)^+$ 28%,
	263 $(M-C_6H_{10}O_2)^+$ 100%, 115 $(M-C_{12}H_{23}NO_5)^+$ 58%.
Analysis; found:-	C, 55.2; H, 9.39; N, 4.04%. $C_{18}H_{35}NO_7$ requires:-
	C, 57.3; H, 9.28; N, 3.71%.

Synthesis of 16-(1-octen-8-yl-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (ON18C6)

This compound was prepared by an analogous synthetic route to that described above from 8-bromooct-1-ene (3.1cm^3, 18mmol), N18C6 (4.0g, 15mmol) and $NaHCO_3$ (1.5g, 18mmol) in DMF (50cm^3). Distillation of the final residue (175°C, 0.1mmHg) gave the product as a clear, pale-yellow oil (3.2g, 56%).

I.R.; ν_{max}	3074 (alkene C-H str) 2928, 2858 (C-H str) 1352 (C-H bend) 1124 (C-O str)
δ_H ($CDCl_3$)	5.78 (1H, d of p, =C*H*) 4.92 (2H, m, =C*H*$_2$, J = 19.4 (trans), 9.7 (cis), 2.4 Hz (gem)) 3.63 (20H, m, -C*H*$_2$-O-C*H*$_2$-) 2.73 (4H, t, -C*H*$_2$-NR-C*H*$_2$-, J = 5.25 Hz) 2.45 (2H, t, >N-C*H*$_2$-, J = 6.0 Hz) 2.00 (2H, q, -C*H*$_2$-CH=, J = 6.0 Hz) 1.35 (8H, m, =CHCH$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-)
δ_C ($CDCl_3$)	139.0 (=CH-) 132.6 (=CH$_2$) 70.7, 70.5, 70.2, 69.6 (-CH$_2$-O-CH$_2$-) 55.9 (-CH$_2$-NR-CH$_2$-) 53.8 (-CH$_2$-N<) 33.6 (-CH$_2$-CH=) 28.9 (-CH$_2$-CH$_2$CH$_2$CH$_2$N<) 28.7 (-CH$_2$-CH$_2$CH=) 27.2 (>NCH$_2$-CH$_2$-) 26.7 (>NCH$_2$CH$_2$-CH$_2$-)
m/e (C.I.)	374 $(M+H)^+$ 100%, 276 $(M-C_7H_{13})^+$ 42%.
Analysis; found :-	C, 64.0; H, 10.9; N, 3.90%. $C_{20}H_{39}NO_5$ requires :- C, 64.3; H, 10.5; N, 3.75%.

Synthesis of 7-(1-hexen-6-yl)-1,4,10-trioxa-7,13-diazacyclopentadecane (HDN15C5)

This compound was prepared as above using 6-bromohex-1-ene (1.8cm^3, 14mmol), DN15C5 (3.0g, 13mmol) and $NaHCO_3$ (1.2g, 14mmol) in DMF (50cm^3). The brown residue after work-up was distilled (180^{o}C, 0.1mmHg), and the oil finally purified using a neutral alumina column (2% MeOH/ DCM) to give a clear, colourless oil (2.3g, 56%).

I.R.; ν_{max}	3329 (N-H str) 3074 (alkene C-H str) 2932, 2856 (C-H str) 1126 (C-O str)
δ_H ($CDCl_3$)	5.80 (1H, d of p, =C*H*-) 4.98 (2H, m, =C*H*$_2$, J = 18.0 (trans), 10.5 (cis) 1.5 Hz (gem)) 3.62 (12H, m, -C*H*$_2$-O-C*H*$_2$-) 2.71 (8H, m, -C*H*$_2$-NR-C*H*$_2$-) 2.50 (2H, t, -C*H*$_2$-N<, J = 6.0 Hz) 2.04 (2H, q, -C*H*$_2$-CH=, J = 6.0 Hz) 1.45 (2H, m, -C*H*$_2$-CH_2N<) 1.35 (2H, m, =$CHCH_2$-C*H*$_2$-)
δ_C ($CDCl_3$)	138.5 (=*C*H-) 114.2 (=*C*H_2) 70.3, 70.1, 69.9, 69.8, 69.7, 69.6, 69.4, 69.3, 69.2 (-*C*H_2-O-*C*H_2-) 56.3 (-*C*H_2-N<) 54.3, 54.3, 54.2, 54.1 (-*C*H_2-NR-*C*H_2-) 33.9 (-*C*H_2-CH=) 26.5 (=$CHCH_2$-*C*H_2-)
m/e (C.I.)	301 $(M+H)^+$ 100%, 219 $(M-C_6H_9)^+$ 69%, 132 $(M-C_{10}H_{18}NO)^+$ 36%.
Analysis; found :-	C, 64.8; H, 11.0; N, 9.00%. $C_{16}H_{32}N_2O_3$ requires :- C, 64.0; H, 10.7; N, 9.33%.

Synthesis of 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane[164].

Under a nitrogen atmosphere a solution of 2,2'-(ethylenedioxy)bis(ethylamine) (7.4g, 50mmol) in MeCN (100cm^3) was added over 2 hours to a boiling solution of triethylene glycol di-*p*-tosylate (20.0g, 43mmol) and finely ground, dried sodium

carbonate (26.5g, 250mmol) in MeCN (400cm^3·). The mixture was then refluxed for 18 hours. Filtration and evaporation of the filtrate gave a residue which was dissolved in a boiling mixture of dioxane (50cm^3) and acetone (50cm^3). The solution was then placed in a freezer and left overnight. The white solid was collected and dissolved in water. The aqueous solution was extracted with chloroform (6×20cm^3). The chloroform was removed *in vacuo* and the solid was recrystallised from heptane to yield a white powder (1.0g, 10%).

I.R.; ν_{max} (nujol)	3320 (N-H str) 1138 (C-O str)
δ_H ($CDCl_3$)	3.61 (16H, m, -C<u>H</u>$_2$-O-C<u>H</u>$_2$-)
	0 (8H, t, -C<u>H</u>$_2$-NH-C<u>H</u>$_2$-, J = 4.2 Hz)
δ_C ($CDCl_3$)	70.3, 70.1 (-<u>C</u>H$_2$-O-<u>C</u>H$_2$-) 49.2 (-<u>C</u>H$_2$-NH-<u>C</u>H$_2$-)
m/e (C.I.)	305 $(M+H+C_3H_6)^+$ 16%, 263 $(M+H)^+$ 100%,
	132 $(M+H-C_6H_{13}NO_2)^+$ 24%.
Analysis; found:-	C, 54.8; H, 10.1; N, 10 6%. $C_{12}H_{26}N_2O_4$ requires:-
	C, 54.9; H, 9.91; N, 10.7%.
Melting point:-	109-111°C

<u>Synthesis of 7,16-(di-1-buten-4-yl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (DBDN18C6)</u>

This product was formed by an analogous procedure to that given above, from 4-bromobut-1-ene (4.0cm^3, 39mmol), DN18C6 (4.0g, 15mmol) and $NaHCO_3$ (3.0g, 36mmol) in DMF (50cm^3). The residue was finally distilled (180°C, 0.05mmHg) to give a clear, colourless oil (3.3g, 60%).

I.R.; ν_{max}	3075 (alkene C-H str) 2950, 2870 (C-H str) 1133 (C-O str)

δ_H ($CDCl_3$)	5.80 (2H, d of p, =C<u>*H*</u>-) 4.98 (4H, m, =C<u>*H*</u>$_2$, J = 17.5 (trans),
	9.0 (cis), 1.6 Hz (gem)) 3.65 (16H, m, -C<u>*H*</u>$_2$-O-C<u>*H*</u>$_2$-)
	2.75 (8H, t, -C<u>*H*</u>$_2$-NR-C<u>*H*</u>$_2$-, J = 6.0 Hz)
	2.58 (2H, t, >N-C<u>*H*</u>$_2$-, J = 6.0 Hz)
	2.19 (4H, q, -C<u>*H*</u>$_2$-CH=, J = 6.0 Hz)
δ_C ($CDCl_3$)	136.6 (=<u>C</u>H-) 115.4 (=<u>C</u>H_2) 70.6, 69.8 (-<u>C</u>H_2-O-<u>C</u>H_2-)
	55.1 (-<u>C</u>H_2-NR-<u>C</u>H_2-) 53.6 (>N-<u>C</u>H_2-) 31.5 (-<u>C</u>H_2-CH=)
m/e (C.I.)	371 $(M+H)^+$ 100%, 329 $(M-C_3H_5)^+$ 86%,
	316 $(M+C_4H_6)^+$ 16%.
Analysis; found:-	C, 64.2; H, 10.2; N, 7.1%. $C_{20}H_{38}N_2O_4$ requires:-
	C, 64.8; H, 10.2; N, 7.5%.

<u>Synthesis of 7,16-(di-1-hexen-6-yl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (DHDN18C6)</u>

An analogous reaction to that above but using 6-bromohex-1-ene (5.0g, 30mmol), DN18C6 (3.6g, 13mmol) and $NaHCO_3$ (2.6g, 30mmol) in DMF (50cm^3) afforded the required product. It was isolated as a pale-yellow oil (3.7g, 63%) following distillation (200^oC, 0.05mmHg).

I.R.; ν_{max}	3074 (alkene C-H str) 2932, 2858 (C-H str) 1639 (C=C str)
	1128 (C-O str)
δ_H ($CDCl_3$)	5.80 (2H, oct, =C<u>*H*</u>-) 4.95 (4H, t, =C<u>*H*</u>$_2$, J = 17.3 (trans),
	10.5 (cis), 0.8 Hz (gem)) 3.60 (16H, m, -C<u>*H*</u>$_2$-O-C<u>*H*</u>$_2$-)
	2.76 (8H, t, -C<u>*H*</u>$_2$-NR-C<u>*H*</u>$_2$-, J = 6.0 Hz)
	2.49 (4H, t, >N-C<u>*H*</u>$_2$-, J = 6.0 Hz)
	2.06 (2H, q, =CH-C<u>*H*</u>$_2$-, J = 6.0 Hz)
	1.40 (8H, m, =CHCH_2-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-CH_2N<)

δ_C ($CDCl_3$)	138.6 (=<u>C</u>H-) 114.2 (=<u>C</u>H_2) 70.5, 70.1, 69.8 (-<u>C</u>H_2-O-<u>C</u>H_2-) 55.5 (-<u>C</u>H_2-NR-<u>C</u>H_2-) 53.7 (-<u>C</u>H_2-N<) 33.4 (=CH-<u>C</u>H_2-) 26.5 (-<u>C</u>H_2-CH_2N<) 26.5 (=$CHCH_2$-<u>C</u>H_2-)
m/e (C.I.)	427 $(M+H)^+$ 97%, 359 $(M+H-C_5H_9)^+$ 44%, 345 $(M+H-C_6H_{11})^+$ 100%.
Analysis; found:-	C, 66.8; H, 10.9; N, 6.70%. $C_{24}H_{36}N_2O_4$ requires:- C, 67.6; H, 10.8; N, 6.57%.

<u>Attempted synthesis of 1,4-dicarbonylbut-2-enyl-1(16),4(16')-bis-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (BisN18C6Fum)</u>

A stirred mixture of N18C6 (1.0g, 3.8mmol) and $NaHCO_3$ (0.3g, 3.8mmol) in MeCN ($20cm^3$) was treated dropwise with fumaryl chloride (0.2g, 1.5mmol) dissolved in MeCN ($20cm^3$). The stirred mixture was held at 50°C for 3 days. The mixture was filtered before the solvent was then removed *in vacuo*. The impure product was distilled from the residue (125°C, 0.1mmHg) and isolated as a clear pale-yellow oil (0.4g).

I.R.; ν_{max}	3584 (O-H) 3053 (alkene C-H str) 2870 (C-H str) 1624 (C=O str) 1118 (C-O str)
δ_H ($CDCl_3$)	5.10 (2H, s, -C<u>H</u>=C<u>H</u>-) 3.58 (48H, m, -C<u>H</u>$_2$-O-C<u>H</u>$_2$-)

Spectroscopy revealed contamination with one or more starting materials, and CHN data were therefore not obtained.

Attempted synthesis of 2-methylene-1,4-dicarbonylbutanyl-1(16),4(16')-bis-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (BisN18C6Ita)

Reaction of N18C6 (2.0g, 7.6mmol), $NaHCO_3$ (0.3g, 3.8mmol) and itaconyl chloride (1.0g, 3.0mmol) in MeCN ($40cm^3$) using the procedure described above yielded after distillation (125°C, 0.1mmHg) a dark-yellow oil (0.4g). This was shown by spectroscopic examination to be an impure sample of the required product so microanalytical data were not obtained.

I.R.; ν_{max}	3142 (alkene C-H str) 2937, 2876 (C-H str) 1635 (C=O str) 1116 (C-O str)
δ_H ($CDCl_3$)	5.12 (2H, d, =C$\underline{H}_2$) 3.58 (50H, m, -C$\underline{H}_2$-O-C$\underline{H}_2$-, C(O)C$\underline{H}_2$-C(=CH_2)C(O)-) 2.72 (4H, m, -C$\underline{H}_2$-NR-C$\underline{H}_2$-)
δ_C ($CDCl_3$)	172.4,171.3 (>$\underline{C}$=O) 170.0 (>C=CH_2) 70.6, 70.5, 70.4, 70.4, 70.3, 70.2, 69.8, 69.5, 69.4, 69.3, 69.2, 68.9 (-$\underline{C}H_2$-O-$\underline{C}H_2$-) 49.0 (-$\underline{C}H_2$-C(=CH_2)-).
m/e (FAB^+)	621.3 $(M+H)^+$ 22%, 358 $(M-C_{12}H_{24}NO_5)^+$ 40%.

Synthesis of 1,8-dicarbonyloctanyl-1(16),8(16')-bis-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (BisN18C6Sub)

The product was isolated as an off-white solid (1.4g, 66%) from a similar reaction procedure to that given above but using N18C6 (2.0g, 7.6mmol), $NaHCO_3$ (0.6g, 7.6mmol) and suberoyl chloride (1.3g, 6.2mmol) in MeCN ($40cm^3$). Distillation was carried out at 125°C and 0.1mmHg pressure.

I.R.; ν_{max}	3584 (O-H str) 2868 (C-H str) 1641 (C=O str) 1120 (C-O str)

δ_H ($CDCl_3$)	3.64 (48H, m, -C<u>H</u>$_2$-O-C<u>H</u>$_2$-, -C<u>H</u>$_2$-NR-C<u>H</u>$_2$-)
	2.34 (4H, t, -C<u>H</u>$_2$-NR_2) 1.63 (4H, s br, -C<u>H</u>$_2$-CH_2NR_2)
	1.34 (4H, s br, -C<u>H</u>$_2$- $CH_2CH_2NR_2$)
δ_C ($CDCl_3$)	173.2 (>N-<u>C</u>O-) 70.7, 70.6, 70.5, 70.5, 70.4, 70.3, 70.2,
	70.1, 70.0, 69.8, 69.4 (-<u>C</u>H_2-O-<u>C</u>H_2-)
	46.6 (-<u>C</u>H_2-NR-<u>C</u>H_2-) 32.9 (-<u>C</u>H_2-C(O)N<)
	29.2 (-<u>C</u>H_2-CH_2CH_2C(O)N<) 25.1 (-<u>C</u>H_2-CH_2C(O)N<)
m/e (FAB^+)	665 $(M+H)^+$ 100%, 402 $(M-C_{12}H_{24}NO_5)^+$ 34%,
	264 $(M+H-C_{20}H_{35}NO_7)^+$ 68%.
Analysis; found :-	C, 57.3; H, 9.00; N, 4.10%. $C_{32}H_{60}N_2O_{12}$ requires :-
	C, 57.8; H, 9.04; N, 4.22%.

<u>Attempted synthesis of 1,3-dicarbonylbenzene-1(16),3(16')-bis-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (BisN18C6Iso)</u>

An analogous reaction to that given above using N18C6 (1.0g, 3.8mmol), $NaHCO_3$ (0.3g, 3.8mmol) and iso-phthaloyl chloride (0.4g, 1.9mmol) in MeCN ($40cm^3$) was attempted. The residue was finally distilled (170°C, 0.1mmHg) to give a yellow oil (0.5g) which was shown to be a slightly impure sample of the required product.

I.R.; ν_{max}	2868 (C-H str) 1633 (C=O str) 1122 (C-O str)
δ_H ($CDCl_3$)	7.42 (4H, m, <u>Ar</u>-) 3.80 (8H, m, -C<u>H</u>$_2$-NR-C<u>H</u>$_2$-)
	3.63 (40H, m, -C<u>H</u>$_2$-O-C<u>H</u>$_2$-)
δ_C ($CDCl_3$)	172.1 (>N-(<u>C</u>=O)-Ar) 136.4 (>N-(C=O)-<u>Ar</u>) 128.4, 127.4,
	125.1 (Ar-<u>C</u>H_2-) 70.7, 70.5, 70.3, 69.4 (-<u>C</u>H_2-O-<u>C</u>H_2-)
	53.3, 49.9, 45.8 (-<u>C</u>H_2-NR-<u>C</u>H_2-)
m/e (FAB^+)	657 $(M+H)^+$ 100%, 394 $(M-C_{12}H_{24}NO_5)^+$ 16%.

Analysis; found :- C, 56.1; H, 7.76; N, 4.20%. $C_{32}H_{52}N_2O_{12}$ requires :-
C, 58.5; H, 7.93; N, 4.27%.

Attempted synthesis of 1,2-dicarbonylbenzene-1(16),2(16')-bis-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (BisN18C6Pht)

Distillation (225°C, 0.1mmHg) of the residue from the reaction of N18C6 (1.0g, 3.8mmol), $NaHCO_3$ (0.3g, 3.8mmol) and phthaloyl chloride (0.4g, 1.9mmol) in MeCN ($40cm^3$), following the procedure described above gave a clear colourless oil in very low yield (30mg) which was shown to be impure by spectroscopy and hence CHN data were not obtained.

I.R.; ν_{max}	3420 (O-H str) 2868 (C-H str) 1630 (C=O str)
	1122 (C-O str)
δ_H ($CDCl_3$)	7.27 (4H, d of p, *Ar*) 3.62 (48H, m, -C*H*$_2$-O-C*H*$_2$-)
δ_C ($CDCl_3$)	170.4 (>*C*=O) 135.8, 134.8, 128.2, 126.4 (*Ar*)
	70.5, 70.4, 70.3, 70.2, 70.1, 69.5, 68.6 (-*C*H$_2$-O-*C*H$_2$-)
	49.6 (>NCH$_2$-*C*H$_2$-O-) 45.0 (>N-*C*H$_2$)
m/e (FAB^+)	657 $(M+H)^+$ 74%, 394 $(M-C_{12}H_{24}NO_5)^+$ 100%.

Synthesis of 1,6-dicarbonylhex-3-enyl-1(16),6(16')-bis-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (BisN18C6Muc)

To a stirred solution of *trans*-hex-3-ene-1,6-dioic acid (0.5g, 3.8mmol) in THF ($30cm^3$) was added HOBT (0.1g, 1.0mmol) and N18C6 (2.0g, 7.6mmol). The mixture was stirred at 0°C for 30 mins. Solid DCC (2.0g, 9.5mmol) was added to the mixture and stirred for a further 5 mins at that temperature. Stirring was then continued at room temperature for a further 3 days. Precipitated DCU was removed by filtering and the solvent removed from the filtrate *in vacuo*. The residue was

dissolved in DCM ($10cm^3$) and the solution refiltered to remove any further solids. The DCM was partially removed *in vacuo* prior to purification using column chromatography (2×2cm, neutral alumina) with an eluant of triethylamine/ DCM/ Petroleum ether (40-60°C) (3:85:12). The crude product was distilled (100°C, 0.05mmHg) to yield an off-white solid (0.8g, 33%).

I.R.; ν_{max}	3505 (O-H str) 2868 (C-H str) 1637 (C=O str) 1120 (C-O str)
δ_H ($CDCl_3$)	5.64 (2H, s, -C<u>*H*</u>=C<u>*H*</u>-) 3.58 (48H, m, -C<u>*H*$_2$</u>-O-C<u>*H*$_2$</u>-, -C<u>*H*$_2$</u>-NR-C<u>*H*$_2$</u>-) 3.12 (4H, d, -C<u>*H*$_2$</u>-CH=CH-C<u>*H*$_2$</u>-)
δ_C ($CDCl_3$)	171.2 (>C=O) 126.4 (-CH=CH-) 70.5, 70.4, 70.1, 69.6, 69.4 (-CH$_2$-O-CH$_2$-) 48.9 (-O-CH$_2$-CH$_2$-N<) 46.7 (-CH$_2$-N<) 36.9 (-CH$_2$-CH=CH-CH$_2$-)
m/e (FAB^+)	635 $(M+H)^+$ 52%, 373 $(M+H-C_{12}H_{24}NO_5)^+$ 3%.
Analysis; found :-	C, 56.7; H, 8.68; N, 4.79%. $C_{30}H_{54}N_2O_{12}$ requires:- C, 56.8; H, 8.20; N, 4.42%.

<u>Synthesis of 1,6-dicarbonylhexanyl-1(16),6(16')-bis-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (BisN18C6Adi)</u>

The preparation of this compound followed the same method as above using N18C6 (2.0g, 7.6mmol), 1,6-hexanedioic acid (0.5g, 3.8mmol), HOBT (0.1g, 1.0mmol) and DCC (2.0g, 9.5mmol) in DMF ($30cm^3$). The residue was distilled (100°C, 0.05mmHg) to give an off-white solid (0.7g, 28%).

I.R.; ν_{max}	2870 (C-H str) 1633 (C=O str) 1120 (C-O str)
δ_H ($CDCl_3$)	3.65 (48H, m, -C<u>*H*$_2$</u>-O-C<u>*H*$_2$</u>-) 2.39 (4H, s, >N-CO-C<u>*H*$_2$</u>-) 1.66 (4H, s, -C<u>*H*$_2$</u>-CH$_2$-CON<)

δ_C ($CDCl_3$)	172.7 (>N-<u>C</u>(O)-) 70.6, 70.5, 70.4, 70.3, 70.2, 70.0, 69.6, 69.2 (-<u>C</u>H_2-O-<u>C</u>H_2-) 48.6 (-O-<u>C</u>H_2-CH_2NRCH_2-<u>C</u>H_2-O-) 46.5 (-<u>C</u>H_2-NR-<u>C</u>H_2-) 32.7 (>N(CO)-<u>C</u>H_2-) 24.8 (>N(CO)-CH_2-<u>C</u>H_2)
m/e (FAB^+)	637 $(M+H)^+$ 52%, 374 $(M+H-C_{12}H_{24}NO_5)^+$ 40%
Analysis; found:-	C, 57.0; H, 8.97; N, 4.79%. $C_{30}H_{56}N_2O_{12}$ requires :- C, 56 6; H, 8.97; N, 4.40%.

<u>Synthesis of 1,4-dicarbonylbut-2-enyl-1[16],4[16']-bis-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (BisN18C6Fum)</u>

A similar reaction was carried out using N18C6 (2.0g, 7.6mmol), fumaric acid (0.4g, 3.8mmol), HOBT (0.1g, 1.0mmol) and DCC (2.0g, 9.5mmol) in THF ($30cm^3$). The residue after work-up was distilled (125°C, 0.1mmHg) to give a yellow solid (1.0g, 43%).

I.R.; ν_{max}	3559 (O-H str) 3065 (alkene C-H str) 2866 (C-H str) 1633 (C=O str) 1120 (C-O str)
δ_H ($CDCl_3$)	7.41 (2H, s, -C<u>H</u>=) 3.64 (48H, m, -C<u>H</u>$_2$-O-C<u>H</u>$_2$-,-C<u>H</u>$_2$-NR-C<u>H</u>$_2$-)
δ_C ($CDCl_3$)	165.3 (><u>C</u>=O) 131.3 (-<u>C</u>H=) 70.6, 70.5, 70.3, 70.2, 69.7, 69.4 (-<u>C</u>H_2-O-<u>C</u>H_2-) 48.9, 47.3 (-<u>C</u>H_2-NR-<u>C</u>H_2-)
m/e.(FAB^+)	607 $(M+H)^+$ 100%, 370 $(M-C_{12}H_{24}NO_5)^+$
Analysis; found :-	C, 55.5; H, 8.25; N, 4.62%. $C_{28}H_{50}N_2O_{12}$ requires :- C, 55.5; H, 8.50; N, 4.68%.

Synthesis of 2-methylene-1,4-dicarbonylbutanyl-1(16),4(16')-bis-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (BisN18C6Ita)

The reactants N18C6 (1.5g, 5.7mmol), itaconic acid (0.4g, 2.7mmol), HOBT (0.1g, 1.0mmol) and DCC (2.0g, 9.5mmol) in THF ($30cm^3$) were treated as described above to yield a light-brown oil (0.5g, 23%) following distillation (100^oC, 0.1mmHg) of the crude product.

I.R.; ν_{max}	3520 (O-H str) 1614 (C=O str) 1115 (C-O str)
δ_H ($CDCl_3$)	6.11 (2H, s, $=C\underline{H}_2$) 3.63 (50H, m, $-C\underline{H}_2$-O-$C\underline{H}_2$-, $-C\underline{H}_2$-C(=CH_2)-)
δ_C ($CDCl_3$)	171.9 (-CH_2-$\underline{C}$(=O)-) 164.8 (>$\underline{C}$(=CH_2)) 146.8 (>C=$\underline{C}H_2$) 71.3, 71.2, 71.1, 71.0, 70.9, 70.4, 70.3, 70.2, 70.1, 69.8, 69.7, 69.4, 69.3, 69.1, 68.8, 68.6, 68.5 (-$\underline{C}H_2$-O-$\underline{C}H_2$-) 48.9, 48.8, 48.6, 48.5 (-$\underline{C}H_2$-NR-$\underline{C}H_2$-) 44.6 (-$\underline{C}H_2$-C(=CH_2)-)
m/e (FAB^+)	621 $(M+H)^+$ 68%, 358 $(M-C_{12}H_{24}NO_5)^+$ 100%
Analysis; found :-	C, 55.9; H, 8.53; N, 4.78%. $C_{29}H_{52}N_2O_{12}$ requires :- C, 56.1; H, 8.38; N, 4.52%.

Synthesis of 1,3-dicarbonylbenzene-1(16),3(16')-bis-1,4,7,10,13-pentaoxa-16-amidocyclooctadecane (BisN18C6Iso)

This reaction was carried out as above using N18C6 (2.0g, 7.6mmol), iso-phthalic acid (0.6g, 3.6mmol), HOBT (0.1g, 1.0mmol) and DCC (2.0g, 9.5mmol) in THF ($30cm^3$). The residue was distilled (125^oC, 0.1mmHg) to give the required product as a light-brown oil (0.9g, 36%).

I.R.; ν_{max}	3559 (O-H str) 2866 (C-H str) 1623 (C=O str) 1120 (C-O str)

δ_H ($CDCl_3$)	7.37 (4H, m, *Ar*) 3.72 (8H, q, -C*H*$_2$-NR-C*H*$_2$-)
	3.59 (40H, m, -C*H*$_2$-O-C*H*$_2$-)
δ_C ($CDCl_3$)	171.1 (>*C*=O) 136.9 (-C(=O)-*Ar*) 128.7, 128.2, 127.3,
	125.0 (*Ar*) 70.5, 70.4, 70.3, 70.1, 69.8, 69.6, 69.2 (-*C*H$_2$-O-
	*C*H$_2$-) 49.7 (-*C*H$_2$-NR-*C*H$_2$-)
m/e (FAB^+)	657 $(M+H)^+$ 100%, 394 $(M-C_{12}H_{24}NO_5)^+$ 14%
Analysis; found :-	C, 58.1; H, 8.20; N, 4.42%. $C_{32}H_{52}N_2O_{12}$ requires :-
	C, 58.5; H, 7.93; N, 4.27%.

Synthesis of 1(16),3(16')-bis(1,4,7,10,13-pentaoxa-16-azacyclooctadecane)xylene (BisN18C6Iso(Red))[165].

Under a nitrogen atmosphere a solution of lithium aluminium hydride (0.5g, 13mmol) in dry diethyl ether (50cm^3) was gently refluxed while BisN18C6Iso (1.0g, 1.5mmol) in dry diethyl ether (20cm^3) was added slowly over 2 hours. The mixture was then refluxed for a further 2 hours. Stirring was continued overnight before water (20cm^3) was added. The mixture was then filtered and the solvent removed *in vacuo*. The residue was dissolved in DCM (10cm^3) and the solution refiltered to remove any further solids. The DCM was partially removed *in vacuo* prior to purification using column chromatography (2×2cm, neutral alumina) with an eluant of triethylamine/ DCM/ petroleum ether (40-60°C) (3:85:12). The residue was distilled (125°C, 0.2mmHg) to yield a light brown oil (0.3g, 29%).

I.R.; ν_{max}	3584 (O-H str) 2864 (C-H str) 1450 (C=C str)
	1352 (C-H str) 1122 (C-O str)
δ_H ($CDCl_3$)	7.14 (4H, s, *Ar*) 3.57 (44H, m, -C*H*$_2$-O-C*H*$_2$-, Ar-C*H*$_2$-N<)
	2.71 (8H, t, -C*H*$_2$-NR-C*H*$_2$-)

δC ($CDCl_3$)	139.3, 132.6 (*Ar*-CH_2) 129.3, 127.9, 127.4 (*Ar*)
	70.7, 70.6, 70.2, 69.8, (-$\underline{C}H_2$-O-$\underline{C}H_2$-) 59.9 (Ar-$\underline{C}H_2$-N<)
	53.6 (-$\underline{C}H_2$-NR-$\underline{C}H_2$-)
m/e (FAB^+)	629 $(M+H)^+$ 62%
Analysis; found :-	C, 61.2; H, 9.26; N, 4.48%. $C_{32}H_{56}N_2O_{10}$ requires :-
	C, 61.1; H, 8.92; N, 4.46%.

Synthesis of 2-(1-hexen-6-yl)-1,4,7,10-tetraoxacyclododecane (H12C4).

For this reaction lithium perchlorate is the templating reagent recommended in the literature [113], however it was replaced by lithium trifluoromethane sulphonate (CF_3SO_3Li), in order to remove the hazards involved when using organic solvents with ionic metal perchlorates.

Lithium trifluoromethane sulphonate (17.5g, 110mmol) and sodium hydroxide pellets (11.6g, 290mmol) were dissolved in DMSO (50cm^3) and the mixture was stirred for 0.3 hour prior to treatment with a solution of oct-7-ene-1,2-diol (7.2g, 50mmol) in DMSO (10cm^3). The mixture was held at 30°C for 20 hours before the dropwise addition of a solution of 1,8-dichloro-3,6-dioxaoctane (9.3g, 50mmol) in DMSO (10cm^3). The reaction mixture was then held at 110-115°C for three days prior to filtration. The cooled filtrate was poured into water (300cm^3) and the light-brown turbid solution extracted with $CHCl_3$ (4×100cm^3). The combined $CHCl_3$ extracts were dried over $MgSO_4$, and the solvent evaporated to yield a dark-brown clear oil. The crude oil was fractionally distilled under reduced pressure (150°C, 0.02mmHg) to give the product as a clear colourless oil (3.1g, 24%).

I.R.; ν_{max}	2930, 2864 (C-H str) 1618 (C=C str)
	1203, 1132 (C-O str).

δ_H ($CDCl_3$)	5.81 (1H, m, =C<u>*H*</u>-) 4.79 (2H, m, C<u>*H*</u>$_2$=, J = 18.0 (trans),
	11.3 (cis), 1.1 Hz (gem)) 4.03 (1H, m, -O-C<u>*H*</u>(CH_2-)CH_2-)
	3.65 (14H, m, -C<u>*H*</u>$_2$-O-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-)
	2.90 (2H, d, =CH-C<u>*H*</u>$_2$-CH_2-) 1.45 (2H, m, -CH-C<u>*H*</u>$_2$-)
	1.35 (2H, m, =$CHCH_2$-C<u>*H*</u>$_2$-) 1.25 (2H, m, -$CHCH_2$-C<u>*H*</u>$_2$-)
δ_C ($CDCl_3$)	132.6 (=<u>C</u>H-) 114.6 (=<u>C</u>H_2) 77.2 (><u>C</u>H-)
	70.6, 67.9 (-<u>C</u>H_2-O-<u>C</u>H_2-) 55.7 (>CH-<u>C</u>H_2-)
	33.6 (-<u>C</u>H_2-CH=) 29.0 (-<u>C</u>H_2-CH_2CH=)
	25.6 (>$CHCH_2$-<u>C</u>H_2-)
m/e (C.I.)	259 $(M+H)^+$ 12%, 127 $(M+H-C_8H_{14}O)^+$ 60%.
Analysis; found :-	C, 64.2 ; H, 10.5%. $C_{14}H_{26}O_4$ requires :-
	C, 65.1 ; H, 10.2%.

<u>Synthesis of 2-(1-hexen-6-yl)-1,4,7,10,13-pentaoxacyclopentadecane (H15C5)</u>

Sodium hydroxide (11.6g, 115mmol) was ground to a powder and added to freshly distilled DMSO (50cm^3) in a nitrogen atmosphere. The stirred solution was then treated with oct-7-ene-1,2-diol (7.2g, 50mmol) in DMSO (10cm^3). The mixture was heated to 30^{o}C for 20 hours before tetraethylene glycol-p-ditosylate (25.0g, 50mmol) in DMSO (50cm^3) was added. The mixture was then stirred at 115^{o}C for a further 3 days. The solution was filtered, and the cooled filtrate treated with water (300cm^3). The aqueous phase was extracted with DCM (5×40cm^3). The DCM extracts were combined and dried over $MgSO_4$, filtered, and the solvent removed *in vacuo*. The clear brown oil was finally distilled (200^{o}C, 0.1mmHg) to give a clear, colourless oil (4.7g, 31%).

I.R.; ν_{max}	3076 (alkene C-H str) 2930, 2860 (C-H str) 1639 (C=C str)
	1130 (C-O str)

δ_H ($CDCl_3$)	5.82 (1H, d of p, =C<u>*H*</u>-) 4.98 (2H, m, =C<u>*H*</u>$_2$, J = 12.0 (trans), 8.0 (cis) 2 Hz (gem)) 3.66 (17H, m, -C<u>*H*</u>$_2$-O-C<u>*H*</u>$_2$-, >C<u>*H*</u>-) 3.50 (2H, p, -O-C<u>*H*</u>$_2$-CH<) 2.05 (2H, d, -C<u>*H*</u>$_2$-CH=) 1.40 (6H, m, >CH-CH_2-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-)
δ_C ($CDCl_3$)	138.7 (=<u>C</u>H-) 114.1 (=<u>C</u>H$_2$) 79.4 (><u>C</u>H-) 70.8, 70.5, 70.4, 70.3, 0.1, 70.0, 69.8 (-<u>C</u>H$_2$-O-<u>C</u>H$_2$-) 28.6 (>CHCH$_2$-<u>C</u>H$_2$-) 24.9 (-<u>C</u>H$_2$-CH_2CH=)
m/e (C.I.)	303 $(M+H)^+$ 34%, 177 $(M+H-C_8H_{14}O)^+$ 100%, 127 $(M+H-C_{10}H_{18}O_2)+$ 27%.
Analysis; found :-	C, 63.4; H, 10.3%. $C_{16}H_{30}O_5$ requires :- C, 63.6; H, 9.93%.

2.3.3. Synthesis of Functionalised Organosiloxanes

<u>Purification of $Me_3Si[OSi(H)Me]_{40}OSiMe_3$</u>

A commercial sample of poly(methylhydridosiloxane) (112.3g, 8.4mmol) was dissolved in a three-fold excess of toluene (340cm^3), and then stirred for one hour with a six-fold excess of methanol (760cm^3). The mixture was allowed to stand for 24 hours. The toluene layer was finally separated and solvent removed under reduced pressure (40°C, 0.1mmHg) to give the product as a clear, colourless oil (100g, 90%).

I.R.; ν_{max}	2966 (C-H str) 2156 (Si-H) 1259 (Si-CH_3 asym and sym str) 1032 (Si-O-Si asym str)
δ_H ($CDCl_3$)	4.79 (40H, s, ≡Si-<u>*H*</u>) 0.26 (120H, s, -OSi-<u>*Me*</u>) 0.21 (6H, s, -OSi-<u>*Me*</u>$_3$)

Synthesis of Model Siloxanes

A mixture of $Me_3SiO[MeSi(H)O]_{40}SiMe_3$ (25.8g, 10mmol), HMDSO (63.3g, 400mmol), TFMSA (0.10%^{v}/w, 0.1g, 0.7mmol) and water (0.01%^{v}/w, 0.01g, 0.6mmol) were equilibrated at 60-65^{o}C under a nitrogen atmosphere for three hours. DMF (0.05g, 0.7mmol) was added and the solution stirred for one hour at 60^{o}C. The resultant mixture was fractionally distilled at atmospheric pressure under a nitrogen atmosphere.

I.R.; ν_{max} 2961, 2901 (C-H str) 2154 (Si-H) 1259 (Si-CH_3 asym and sym str) 1047 (Si-O-Si asym str)

N.m.r data showed a mixture of tri- and tetra-siloxanes which could not be separated completely by careful fractional distillation. As a gift of a commercial sample of the trisiloxane became available, no further attempts to fractionate the mixture were made.

Synthesis of 1-[(13-hexan-1-yl)-1,4,7,10-tetraoxa-13-azacyclopentadecane]-1,1,1,3,3,5,5-heptamethyltrisiloxane (HN15C5MST)

A mixture of 1,1,1,3,3,5,5-heptamethylhydridotrisiloxane (2.0g, 9.0mmol) and HN15C5 (1.35g, 4.5mmol) was added under a nitrogen atmosphere to dry toluene (2cm^3). A stream of dry nitrogen gas was bubbled through the solution for 30 minutes after which time $C_8H_{12}PtCl_2$ (1mg, 2.6μmol) in DCM (1cm^3) was added. The reaction vessel was immediately sealed and heated at 80^{o}C for 24 hours. A further aliquot of catalyst was added to the cooled solution and the vessel was resealed and heated at 80^{o}C for another 48 hours. The vessel was finally opened and all volatiles were removed *in vacuo*, and the residue washed with methanol (10cm^3).

The filtrate was separated and solvent was removed *in vacuo*. The resulting oil was filtered to give a clear brown oil (1.7g, 73%).

I.R.; ν_{max}	2928, 2858 (C-H str) 1253 (Si-CH_3 str) 1130 (C-O str) 1049 (Si-O-Si str)
δ_H ($CDCl_3$)	3.61 (16H, m, -C<u>*H*</u>$_2$-O-C<u>*H*</u>$_2$-) 2.71 (4H, t, -C<u>*H*</u>$_2$-NR-C<u>*H*</u>$_2$) 2.42 (2H, t, >N-C<u>*H*</u>$_2$-) 1.40 (2H, q, >NCH_2-C<u>*H*</u>$_2$-) 1.30 (6H, m, >NCH_2CH_2-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-) 0.04 (2H, t, -OSi(Me_2)C<u>*H*</u>$_2$-) 0.00 (21H, m, <u>*Me*</u>$_3$-SiO-, --OSi(<u>*Me*</u>$_2$)-, -OSi(<u>*Me*</u>$_2$)CH_2-)
δ_C ($CDCl_3$)	70.9, 70.3, 70.0, 69.9 (-<u>C</u>H_2-O-<u>C</u>H_2-) 57.1 (-<u>C</u>H_2-NR-<u>C</u>H_2) 54.5 (>N-<u>C</u>H_2-) 33.3 (>NCH_2-<u>C</u>H_2-) 27.1 (>NCH_2CH_2-<u>C</u>H_2-) 21.0 (>$NCH_2CH_2CH_2$-<u>C</u>H_2-) 18.1 (OSi(Me_2)CH_2-<u>C</u>H_2-) 1.7, 1.6, 1.2, 0.1 (<u>*Me*</u>$_3$SiO, -OSi(<u>*Me*</u>$_2$), -OSi(<u>*Me*</u>$_2$)<u>C</u>H_2-)
δ_{Si} ($CDCl_3$)	-21.0 (-O<u>*Si*</u>(Me_2)-), +7.0 (-O<u>*Si*</u>(Me_2)CH_2-), +7.5 (Me_3<u>*Si*</u>O-).
Analysis; found :-	C, 51.2; H, 10.1; N, 2.70%. $C_{23}H_{53}NO_6Si_3$ requires :- C, 52.8; H, 10.1; N, 2.68%.

<u>Synthesis of 1-[(16-butan-1-yl)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane]-1,1,1,3,3,5,5-heptamethyltrisiloxane (BN18C6MST)</u>

This compound was prepared by an analogous procedure to that above from 1,1,1,3,3,5,5-heptamethylhydridotrisiloxane (1.0g, 2.3mmol) and BN18C6 (0.7g, 2.3mmol). The product was isolated as a clear, pale-yellow oil (0.8g, 66%).

I.R.; ν_{max}	2926, 2860 (C-H str) 1252 (Si-CH_3 str) 1128 (C-O str) 1049 (Si-O-Si str)

δ_H ($CDCl_3$)	3.60 (20H, m, -C<u>*H*</u>$_2$-O-C<u>*H*</u>$_2$-) 2.77 (4H, t, -C<u>*H*</u>$_2$-NR-C<u>*H*</u>$_2$-)
	2.49 (2H, t, >N-C<u>*H*</u>$_2$-) 1.45 (2H, p, >NCH_2-C<u>*H*</u>$_2$-)
	1.24 (2H, m, >NCH_2CH_2-C<u>*H*</u>$_2$-) 0.43 (2H, m, $OSi(Me_2)C$<u>*H*</u>$_2$)
	0.00 (21H, m, <u>*Me*</u>$_3SiO$, -OSi(<u>*Me*</u>$_2$)-, -OSi(<u>*Me*</u>$_2$)-CH_2-)
δ_C ($CDCl_3$)	70.7, 70.6, 70.2, 69.4 (-<u>C</u>H_2-O-<u>C</u>H_2-) 55.5 (-<u>C</u>H_2-NR-<u>C</u>H_2-) 53.8 (>N-<u>C</u>H_2-) 21.0 (>NCH_2-<u>C</u>H_2-)
	18.1 ($OSi(Me_2)CH_2$-<u>C</u>H_2-) 1.7, 1.6, 1.1, 0.06 (<u>*Me*</u>$_3SiO$-, -OSi(<u>*Me*</u>$_2$)-, -O-Si(<u>*Me*</u>$_2$)<u>C</u>H_2-
δ_{Si} ($CDCl_3$)	-20.9 (-O<u>*Si*</u>(Me_2)-), +7.1 (-O<u>*Si*</u>$(Me_2)CH_2$-), +7.3 (Me_3<u>*Si*</u>O-)
Analysis; found :-	C, 50.4; H, 10.1; N, 2.60%. $C_{23}H_{53}NO_7Si_3$ requires :-
	C, 51.2; H, 9.92; N, 2.60%.

<u>Synthesis of 3-[2(hexan-6-yl)-1,4,7,10,13-pentaoxacyclopentadecane]-1,1,1,3,5,5,5-heptamethyltrisiloxane (H15C5MSM)</u>

The same procedure as above but using 1,1,1,3,5,5,5-heptamethyl-hydridotrisiloxane (1.0g, 2.3mmol) and H15C5 (1.4g, 2.3mmol) gave the product as a clear, pale-yellow oil (1.6g, 65%).

I.R.; ν_{max}	2926, 2868 (C-H str) 1259 (Si-CH_3 str) 1130 (C-O str)
	1049 (Si-O-Si str)
δ_H ($CDCl_3$)	3.65 (19H, m, -C<u>*H*</u>$_2$-O-C<u>*H*</u>$_2$-, >C<u>*H*</u>-O-) 1.35 (10H, m,
	>CH-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-) 0.4 (2H, br s,
	-$OSi(Me_2)C$<u>*H*</u>$_2$-) 0.00 (21H, m, <u>*Me*</u>$_3SiO$, OSi(<u>*Me*</u>)CH_2-)
δ_C ($CDCl_3$)	80.1 (>C<u>H</u>-) 71.4, 71.1, 71.0, 70.9, 70.4 (-<u>C</u>H_2-O-<u>C</u>H_2-)
	33.5 (>CH-<u>C</u>H_2-) 32.4 (>$CHCH_2$-<u>C</u>H_2-)
	29.8 (>$CHCH_2CH_2$-<u>C</u>H_2-) 23.4 (-$OSi(Me)CH_2$-<u>C</u>H_2-)
	1.8, 0.00 (<u>*Me*</u>$_3SiO$, -OSi(<u>*Me*</u>)<u>C</u>H_2-)

δ_{Si} ($CDCl_3$) +6.8 (-O*Si*(Me)CH_2-, Me_3*Si*O-)

Analysis; found :- C, 53.8; H, 10.1%. $C_{23}H_{55}O_7Si_3$ requires :- C, 52.7; H, 9.92%.

Synthesis of methyl[(13(4-butan-1-yl)-1,4,7,10-tetraoxa-13-azacyclopentadecane]siloxane-dimethylsiloxane copolymer (4:96) (BN15C5PS)

This preparation was carried out as above using methylhydro-dimethyl-siloxane copolymer (4:96)(15.2g, 1.2mmol) and BN15C5 (2.5g, 9.1mmol). The product was isolated as a turbid, pale-yellow fluid (15.1g, 85%).

I.R.; ν_{max} 2963, 2905 (C-H str) 1261 (Si-Me_3 str) 1095 (C-O str) 1022 (Si-O-Si str) 802 (Si-O str)

δ_H ($CDCl_3$) 3.65 (128H, m, -C*H*$_2$-O-C*H*$_2$-) 2.78 (32H, d of t, -C*H*$_2$-NR-C*H*$_2$-) 2.50 (16H, t, >N-C*H*$_2$-) 1.50 (16H, d of t, >NCH_2-C*H*$_2$-) 1.42 (16H, d of t, -OSi(Me)CH_2-C*H*$_2$-) 1.00 (16H, q, -OSi(Me)C*H*$_2$-) 0.00 (1074H, m, *Me*$_3$SiO-, -OSi(*Me*$_2$)-, -OSi(*Me*)C*H*$_2$-)

δ_C ($CDCl_3$) 70.9, 70.8, 70.7, 70.3, 70.0 (-CH_2-O-CH_2-) 55.1 (-CH_2-NR-CH_2-) 53.6 (>N-CH_2-) 21.4 (>NCH_2-CH_2-) 18.0 (>NCH_2CH_2-CH_2-) 0.00 (*Me*$_3$SiO-, -OSi(*Me*$_2$)-, -OSi(*Me*)CH_2-)

δ_{Si} ($CDCl_3$) -22.0 (O*Si*(Me$_2$)-)

Analysis; found :- C, 35.1; H, 8.47; N, 0.69%. $C_{500}H_{1296}N_8O_{219}Si_{182}$ requires :- C, 36.1; H, 7.80; N, 0.67%.

Synthesis of 3-allylphenyl ether-1,1,1,3,5,5,5-heptamethyltrisiloxane (APEMSM)

By using 1,1,1,3,5,5,5-heptamethylhydridotrisiloxane (0.5g, 2.3mmol) and allylphenyl ether (0.3g, 2.3mmol) in the procedure detailed above a clear, colourless oil was isolated (0.5g, 61%).

I.R.; ν_{max}	3042 (Ar-H str) 2959, 2903 (C-H str) 1045 (Si-O-Si str)
δ_H ($CDCl_3$)	7.27 (2H, m, Ar-*H* ortho to -CH_2O-) 7.00 (3H, m, Ar-*H* meta and para to -CH_2O-) 3.98 (2H, m, -C*H*$_2$-OAr) 1.88 (2H, q, -C*H*$_2$-CH_2-O-) 0.50 (2H, d of t, -OSi(Me)C*H*$_2$-) 0.00 (21H, m, *Me*$_3$SiO-,-OSi(*Me*)CH_2-)
δ_C ($CDCl_3$)	159.4,129.8, 129.7, 120.7, 114.9, 114.8 (-O-*Ar*) 70.4 (-*C*H_2-O-) 23.3 (-*C*H_2-CH_2O-) 13.8 (-*C*H_2-CH_2CH_2O-)

Synthesis of methyl[2-(1-hexan-6-yl)-1,4,7,10,13-pentaoxacyclopentadecane] siloxane-dimethylsiloxane copolymer (4:96) (H15C5PS)

The same procedure as above was followed using methylhydro-dimethyl-siloxane copolymer (4:96) (2.8g, 200μmol) and H15C5 (0.5g, 1.7mmol). The product was formed as a clear, pale-yellow oil (1.7g, 52%).

I.R.; ν_{max}	2963 (C-H str) 1261 (Si-CH_3 str) 1095 (C-O str) 1020 (Si-O-Si str)
δ_H ($CDCl_3$)	3.65 (136H, m, >C*H*-,-C*H*$_2$-O-C*H*$_2$-) 3.45 (16H, m, >CH-C*H*$_2$-O-) 1.60 (16H, d of d, >CH-C*H*$_2$-) 1.42 (16H, br s, >$CHCH_2$-C*H*$_2$-) 1.31 (16H, br s, >$CHCH_2CH_2$-C*H*$_2$-) 0.00 (1074H, m, *Me*$_3$SiO, -OSi(*Me*$_2$)-, -OSi(*Me*)C*H*$_2$-)

δ_C ($CDCl_3$)	70.9, 70.5, 70.4, 69.9 (-$\underline{C}H_2$-O-$\underline{C}H_2$-, >$\underline{C}$H-CH_2O-)
	34.2 (>CH-$\underline{C}H_2$) 32.1 >$CHCH_2$-$\underline{C}H_2$-)
	29.8 (>$CHCH_2CH_2CH_2$-$\underline{C}H_2$-) 18.4 (-OSi(Me)CH_2-$\underline{C}H_2$-)
	0.00 (-OSi($\underline{Me}_2$)-, $\underline{Me}_3$SiO-, -OSi($\underline{Me}$)$\underline{C}H_2$-)
δ_{Si} ($CDCl_3$)	-21.8 (-O$\underline{Si}$(Me_2))
Analysis; found :-	C, 36.1; H, 8.44%. $C_{486}H_{1322}O_{221}Si_{182}$ requires :-
	C, 36.9; H, 8.37%.

Synthesis of methyl[16-(hexan-6-yl)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane]siloxane-dimethylsiloxane copolymer (4:96) (HN18C6PS)

This copolymer was isolated by the same procedure as above using methylhydro-dimethylsiloxane copolymer (4:96) (2.8g, 200μmmol) and HN18C6 (0.5g, 1.7mmol). The product was obtained as a clear, pale-yellow oil (1.7g, 51%).

I.R.; ν_{max}	2963 (C-H str) 1259 (Si-Me str) 1091 (C-O str)
	1020 (Si-O-Si str)
δ_H ($CDCl_3$)	3.65 (20H, m, -C$\underline{H}_2$-O-C$\underline{H}_2$-) 2.70 (4H, br s, -C$\underline{H}_2$-NR-C$\underline{H}_2$-) 2.51 (2H, br s, >N-C$\underline{H}_2$-) 1.90 (2H, br s, >NCH_2-C$\underline{H}_2$-) 1.45 (2H, br s, >NCH_2CH_2-C$\underline{H}_2$-) 1.32 (4H, m, --OSi(Me)CH_2-C$\underline{H}_2C\underline{H}_2$-) 1.00 (2H, t, -OSi(Me)C$\underline{H}_2$-)
	0.00 (1074H, m, $\underline{Me}_3$SiO-, -OSi($\underline{Me}_2$)-, -OSi($\underline{Me}$)CH_2-)
δ_C ($CDCl_3$)	71.3, 71.2, 70.8, 70.4 (-$\underline{C}H_2$-O-$\underline{C}H_2$-, >N-$\underline{C}H_2$-)
	54.4 (>NCH_2-$\underline{C}H_2$-) 33.8 (>NCH_2CH_2-$\underline{C}H_2$-)
	27.8 (-OSi(Me)CH_2CH_2-$\underline{C}H_2$-) 23.5 (-OSi(Me)CH_2-$\underline{C}H_2$-)
	0.00 (-OSi($\underline{Me}_2$)-, $\underline{Me}_3$SiO-, -OSi($\underline{Me}$)$\underline{C}H_2$-)

δ_{Si} ($CDCl_3$)	-21.9 (-O*Si*(Me_2)-) -21.6 (-O*Si*(Me)CH_2-)
Analysis; found :-	C, 35.7; H, 8.43; N, 0.55%. $C_{466}H_{1362}N_8O_{221}Si_{182}$
	requires :- C, 35.6; H, 8.67; N, 0.71%.

2.3.4. Miscellaneous Reactions

Attempted synthesis of 16-prop-3-ane-1,2-diol-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (PDIOLN18C6)

3-bromopropane-1,2-diol (0.7g, 4.5mmol) was added dropwise to a stirred solution of N18C6 (1.0g, 3.8mmol) and $NaHCO_3$ (0.4g, 5.0mmol) in DMF (30cm^3). The stirred mixture was heated at 50°C for 3 days, then poured into water (20cm^3) and extracted with DCM (4×30cm^3). The extracts were combined and the solvent removed *in vacuo*. The residue was distilled (150°C, 0.01mmHg) to give a yellow viscous oil (0.1g, 8%).

I.R.; ν_{max}	3314 (O-H str) 2876 (C-H str) 1095 (C-O str)
δ_H ($CDCl_3$)	4.80 (1H, oct, >C*H*-OH) 4.52 (2H, sx, -CH_2-OH)
	4.21 (1H, br s, -O*H*) 3.88 (1H, d, -O*H*) 3.62 (20H, m, -
	CH_2-O-CH_2-) 2.80 (4H, t, -CH_2-NR-CH_2-)
δ_C ($CDCl_3$)	70.1, 69.9, 69.4 (-CH_2-O-CH_2-, -CH_2-OH)
	76.3 (>CH-OH) 65.9 (>N-CH_2-) 48.6 (-CH_2-NR-CH_2-)
m/e (C.I.)	338 $(M+H)^+$ 23%, 263 $(M-C_3H_7O_2)^+$ 100%.
Analysis; found :-	C 50.2,; H, 3.65; N, 8.50%. $C_{15}H_{31}NO_7$ requires :-
	C 54.7,; H, 4.26; N, 9.12%

Attempted synthesis of 16-(6-hexane-1,2-diol)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (HDIOLN18C6)

Using a modification of the method by Campbell et al[166] silver acetate (0.5g, 28.8mmol) and iodine (0.2g, 144.0mmol) were stirred at room temperature in glacial acetic acid (40cm^3) until all the iodine was consumed. HN18C6 (0.5g, 144mmol) in glacial acetic acid (20cm^3) was then introduced. The mixture was heated to 50^{o}C and then treated with glacial acetic acid (5cm^3) containing water (80μl). The reaction mixture was stirred at 50^{o}C for a further 5 hours and then filtered to remove the yellow precipitate and the solvent was removed from the filtrate *in vacuo*. The residue was distilled (225^{o}C, 0.01mmHg) to give a clear, pale-yellow oil (0.05g,) which spectroscopy revealed to consist of a complex mixture of products. No microanalytical data were obtained

I.R.; ν_{max}	3451 (O-H str) 3074 (alkene C-H str) 2930, 2862 (C-H str) 1122 (C-O str)
δ_H ($CDCl_3$)	4.10 (1H, m, >C*H*-OH) 3.67 (22H, m, -C*H*$_2$-O-C*H*$_2$-, -C*H*$_2$-OH) 2.78 (4H, d of p, -C*H*$_2$-NR-C*H*$_2$-) 2.55 (2H, d of p, >N-C*H*$_2$-) 1.35 (6H, m, >CH-C*H*$_2$-C*H*$_2$-C*H*$_2$-)
δ_C ($CDCl_3$)	77.6 (>CH-OH) 72.7 (-CH$_2$-OH) 70.4,70.3 (-CH$_2$-O-CH$_2$-) 55.2 (>N-CH$_2$-) 53.9 (-CH$_2$-NR-CH$_2$-) 33.7 (-CH$_2$-CH<) 25.1 (>NCH$_2$-CH$_2$-) 22.6 (>CHCH$_2$-CH$_2$-)

Attempted synthesis of 7-(6-hexan-1,2-diol)-16-(1-hexen-6-yl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (HDIOLHDN18C6)

A mixture of silver acetate (1.5g, 8.6mmol) and iodine (1.1g, 4.3mmol) in glacial acetic acid ($40cm^3$) was stirred at room temperature until all the iodine had been consumed. A solution of DHDN18C6 (1.8g, 4.3mmol) in glacial acetic acid ($25cm^3$) was next introduced. The mixture was then heated to 50°C before the addition of glacial acetic acid ($5cm^3$) containing water (80µl). The mixture was then filtered and the solvent removed from the filtrate *in vacuo*. The residue was passed through a neutral alumina column using gradient elution (0-10% MeOH/ DCM). The appropriate fractions were combined and the solvent removed *in vacuo*. The resulting residue was distilled (150°C, 0.1mmHg) to give a yellow oil (0.2g), which spectroscopy revealed to consist of a complex mixture of products. No microanalytical data were obtained.

I.R.; ν_{max} 3358 (O-H str) 3074 (alkene C-H str) 2934, 2864 (C-H str) 1126 (C-O str)

δ_H ($CDCl_3$) 5.80 (1H, m, =C<u>*H*</u>-) 4.95 (2H, t, =C<u>*H*</u>$_2$, J = 18.8 (trans), 9.0 (cis), 1.5 Hz (gem)) 4.05 (2H, m, >C<u>*H*</u>-OH, -C<u>*H*</u>$_2$-OH) 3.60 (21H, m, -C<u>*H*</u>$_2$-O-C<u>*H*</u>$_2$-) 2.85 (11H, m, -C<u>*H*</u>$_2$-NR-C<u>*H*</u>$_2$-) 2.53 (6H, p, >N-C<u>*H*</u>$_2$) 2.21 (2H, q, -C<u>*H*</u>$_2$-CH=) 2.10 (5H, m, CH_3CO_2H) 1.42 (12H, m, =CHCH$_2$-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-)

Attempted synthesis of 7-(6-hexane-1,2-diol)-16-(1-hexen-6-yl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (HDIOLHDN18C6)

A stirred solution of DHDN18C6 (1.5g, 3.5mmol) in acetone ($10cm^3$) was cooled to -10°C and treated over 3 hours with a solution of $KMnO_4$ (0.6g, 4.0mmol) in 10% aqueous acetone. The mixture was filtered after standing for 1 hour and the filtrate saturated with carbon dioxide. Solvent was then removed *in vacuo*. The resultant oil was redissolved in dry acetone and treated with more carbon dioxide and refiltered. The solvent was again removed from the filtrate *in vacuo* and the residue was distilled (200°C, 0.07mmHg) to give a dark-yellow oil (0.3g), which was shown to be a complex mixture.

I.R.; ν_{max}	3329 (O-H str) 3074 (alkene C-H str) 2932, 2860 (C-H str) 1124 (C-O str)
δ_H ($CDCl_3$)	5.80 (1H, m, =C<u>*H*</u>-) 4.95 (2H, t, =C<u>H_2</u>, J = 18.0 (trans), 10.5 (cis), 2.2 Hz (gem)) 3.65 (10H, m, -C<u>H_2</u>-O-C<u>H_2</u>-) 2.77 (4H, m, -C<u>H_2</u>-NR-C<u>H_2</u>-) 2.53 (2H, t, >N-C<u>H_2</u>-) 2.06 (2H, q, =CH-C<u>H_2</u>-) 1.45 (4H, m, =CHCH$_2$-C<u>H_2</u>C<u>H_2</u>-)

Synthesis of dichloro(1,5-cyclooctadiene)platinum(II)

Following the literature method of Drew et al[163] hydrated chloroplatinic acid (0.9g, 2.2mmol) was dissolved in glacial ethanoic acid ($3.0cm^3$, 52mmol) in a $50cm^3$ Erlenmeyer flask giving a clear, orange solution. The solution was heated to 75°C and treated with 1,5-cyclooctadiene ($1.2cm^3$, 2.2mmol) which produced a black, oily suspension. The reaction mixture was swirled gently and cooled to room temperature. Water ($10cm^3$) was added and the black suspension was stirred for one hour. The crude, grey product was collected on a Buchner funnel, and was washed with water ($10cm^3$) and diethyl ether ($20cm^3$). It was suspended in DCM ($80cm^3$)

and the mixture boiled for five minutes. The solution was cooled, mixed with chromatographic-grade silica gel and allowed to settle. The mixture was then filtered and the residue was a washed with two portions of DCM ($10cm^3$). Solvent was evaporated until the product started to crystallise. The hot solution was poured into petroleum ether (b.p. 60-80°C) to precipitate the white product, which was washed with petroleum ether ($20cm^3$) and dried (0.2g, 23%).

δ_H ($CDCl_3$)	5.59 (4H, t, -C$\underline{H}$=) 2.67 (4H, m, -C$\underline{H}_2$-)
	2.25 (4H, m, -C$\underline{H}_2$-)
Analysis; found:-	C, 25.7 ; H, 3.25%. $C_8H_{12}PtCl_2$ requires :-
	C, 25.7 ; H, 3.23%.

Preparation of 1,11-dichloro-3,6,9-trioxaundecane

Tetraethylene glycol ($38.5cm^3$, 200mmol), toluene ($200cm^3$) and pyridine ($40cm^3$) were heated together to 86°C and thionyl chloride ($36cm^3$, 500mmol) was added dropwise with stirring over one hour. Heating was continued overnight (16 hours) during which time a pale-yellow layer had separated from a lower layer consisting of a red viscous oil. Hydrochloric acid (20%v/v, $25cm^3$) was added dropwise to the mixture, and the upper toluene layer containing the product was removed. The toluene was evaporated *in vacuo* to give the product as a pale-yellow liquid (50.3g, 95%).

I.R.; ν_{max}	2959, 2872 (C-H str) 1300, 1120 (C-O str)
δ_H ($CDCl_3$)	3.70 (4H, m, Cl-C$\underline{H}_2$-, J = 4.3 Hz) 3.73 (4H, m, Cl-CH_2-C$\underline{H}_2$-) 3.82 (8H, d of t, O-C$\underline{H}_2$-C$\underline{H}_2$-O-, J = 5.4 Hz)
Analysis; found :-	C, 42.9 ; H, 7.12%. $C_8H_{16}Cl_2O_3$ requires :-
	C, 41.5 ; H, 6.99%.

Synthesis of di-(2-hydroxyethyl)allylamine

Following the method described by Ford-Moore et al[167] allyl bromide (15.0g, 110mmol) was added to an ice-cooled mixture of diethanolamine (15.0g, 140mmol) and anhydrous sodium carbonate (10.0 g, 90mmol).The reaction mixture was then warmed to 60°C on a water bath for two hours. Ethanol (50cm^3) was added to the mixture, which was then filtered and distilled (120°C, 0.1mmHg) to give the product as a clear, colourless oil (6.4g, 36%).

I.R.; ν_{max}	3354 (O-H str) 2943, 2882, 2831 (C-H str) 1643 (C=C str) 1041 (C-O str)
δ_H ($CDCl_3$)	5.86 (1H, m, CH_2=C<u>*H*</u>-) 5.21 (2H, br. s, -O<u>*H*</u>)
	5.15 (2H, m, C<u>*H*</u>$_2$=, J = 16.0 (trans), 9.0 (cis), 1.0 Hz (gem))
	3.69 (4H, d of t, HO-C<u>*H*</u>$_2$-) 3.2 (2H, d, >N-C<u>*H*</u>$_2$-CH=)
	2.65 (4H, m, >N-C<u>*H*</u>$_2$-CH_2-)
m/e (E.I.)	145 (M^+) 3%, 114 $(M\text{-}CH_3O)^+$ 100%, 100 $(M\text{+-}C_2H_5O)^+$ 3%, 41 $(M\text{-}C_4H_{10}NO_2)$+ 52%.
Analysis; found :-	C, 54.8; H, 10.5; N, 9.95%. $C_7H_{15}NO_2$ requires :- C, 57.9; H, 10.41; N, 9.65%.

Synthesis of 3-bromo-propan-1,2-diol

A stirred solution of 3-bromoprop-1-ene (10.0g, 83mmol) in acetone (30cm^3) was cooled to -10°C using an ice/ salt bath and treated dropwise with a solution of $KMnO_4$ (15.8g, 100mmol) in 10% aqueous acetone (300cm^3). The mixture was then stirred for an hour and filtered. The clear, pale-yellow filtrate was saturated with solid carbon dioxide and then refiltered. Solvent was removed *in vacuo* to give a pale-yellow oil which was redissolved in dry acetone and treated again with carbon dioxide. The mixture was filtered and solvent once again removed *in vacuo*. The

remaining oil was dissolved in DCM ($10cm^3$) and dried over $MgSO_4$. The residue remaining after removal of the solvent was distilled (80^oC, 0.05mmHg) to give a clear, colourless oil (2.6g, 20%).

I.R.; ν_{max}	3356 (O-H str) 2934 (C-H str) 1063 (C-O str)
δ_H ($CDCl_3$)	3.98 (1H, m, >C<u>*H*</u>-OH) 3.81 (2H, m, -C<u>H_2</u>-OH)
	3.47 (2H, m, -C<u>H_2</u>-Br)
δ_C ($CDCl_3$)	71.4 (><u>C</u>H-OH) 64.2 (-<u>C</u>H_2-OH) 34.4 (-<u>C</u>H_2-Br)
m/e (C.I.)	157 $(^{(81Br)}M+H)^+$ 14%, 155 $(^{(79Br)}M+H)^+$ 15%,
	139 $(^{(81Br)}M-HO)^+$ 97%, 137 $(^{(79Br)}M-HO)^+$ 100%.
Analysis, found :-	C, 23.5; H, 4.72%. $C_3H_7BrO_2$ requires :-
	C, 23.2; H, 4.52%.

<u>Synthesis of triethylene glycol ditosylate</u>

Following the literature preparation of Dale et al[168] triethylene glycol (15.0g, 100mmol) was dissolved in dry pyridine ($80cm^3$) and powdered *p*-toluenesulphonyl chloride (38.5g, 200mmol) added under a nitrogen atmosphere portion-wise over two hours to the stirred solution maintained at 0^oC. Stirring and cooling was continued for a further four hours and then the mixture was left stirring at room temperature overnight. The reaction mixture was poured onto ice (100g) and further diluted with water ($50cm^3$). The precipitated product was collected and washed on the filter with water ($160cm^3$). Recrystallisation from ethanol ($200cm^3$) gave the pure product as a white crystalline powder (10.8g, 24%).

I.R.; ν_{max}	1597 (C=C str) 1176 (C-O str)
δ_H ($CDCl_3$)	7.79 (4H, d of d, -<u>*Ar*</u>-SO_2-) 7.35 (4H, d of d, C<u>H_3</u>-Ar-)
	4.14 (4H, t, -SO_2-O-C<u>H_2</u>-) 3.64 (4H, q, -SO_2-O-C<u>H_2</u>-

	CH_2-O-, J=4.5 Hz) 3.52 (4H, s, O-C$\underline{H}_2$-C$\underline{H}_2$-O-, J=4.5 Hz)
	2.44 (6H, s, C$\underline{H}_3$-Ar)
δC ($CDCl_3$)	129.8 ($\underline{Ar}$-SO_3-) 127.9 (CH_3-$\underline{Ar}$) 70.6 (-SO_3-$\underline{C}H_2$-)
	69.1 (-SO_3-CH_2-$\underline{C}H_2$-O-) 68.6 (-O-$\underline{C}H_2$-$\underline{C}H_2$-O-)
	21.5 ($\underline{C}H_3$-)
m/e (C.I.)	459 $(M-H)^+$ 31%, 323 $(M+H-C_7H_7SO)^+$ 100%.
Analysis; found :-	C, 52.2; H, 5.77%. $C_{20}H_{26}O_8S_2$ requires :-
	C, 52.2; H, 6.08%.

Synthesis of tetraethylene glycol ditosylate

p-Toluene sulphonyl chloride (57.8g, 300mmol) was added over a 2 hour period to a stirred solution of tetraethylene glycol (29.2g, 150mmol) in ice-cold pyridine (120cm^3). The reaction mixture was allowed to stir overnight, then it was poured onto ice (150g) and diluted with water (75cm^3). The solution was then made acidic with hydrochloric acid (18%) and extracted with chloroform (4×20cm^3). The extracts were combined, washed with saturated sodium carbonate solution (50cm^3) and then with water (50cm^3) and dried over $MgSO_4$. The solvent was removed from the filtrate *in vacuo* to give a clear, pale-yellow oil (36.0g, 48%).

I.R.; ν_{max}	2870 (C-H str) 1358 (C=C str) 1120 (C-O str)
δ_H ($CDCl_3$)	7.78 (4H, q, $\underline{Ar}$-SO_2-) 7.33 (4H, q, $\underline{Ar}$-Me)
	4.14 (4H, q, -SO_2O-C$\underline{H}_2$-) 3.64 (12H, m, -C$\underline{H}_2$-O-C$\underline{H}_2$-)
	2.44 (6H, s, $\underline{Me}$-Ar)
δ_C ($CDCl_3$)	144.6, 132.7, 129.6, 129.3, 127.7, 127.4 (-$\underline{Ar}$-)
	71.1 (-SO_2O$\underline{C}H_2$-CH_2-) 70.5 (-SO_2O-$\underline{C}H_2$-)
	70.4, 70.3 (-$\underline{C}H_2$-O-$\underline{C}H_2$-) 21.4 ($\underline{Me}$-Ar)
m/e (FAB^+)	503 $(M+H)^+$ 24%, 199 $(M-C_{13}H_{19}O_6S)^+$ 40%.
Analysis; found :-	C, 53.2; H, 6.01%. $C_{22}H_{30}O_9S_2$ requires :-
	C, 52.3; H, 6.02%.

Chapter Three

Analytical Techniques

3.1. Summary

The modified crown ether analogues, the functionalised siloxanes and the precursors described in the previous chapter were fully characterised chemically using a range of analytical techniques. These included, where appropriate, a combination of infra-red and nuclear magnetic resonance (n.m.r) spectroscopies, microanalysis and mass spectroscopy. High performance liquid chromatography (HPLC) was also used to assess the effectiveness of the functionalised polysiloxanes for amino-acid complexation by determining the amino-acid concentrations before and after extractions.

3.2. Infra-red Spectroscopy

Infra-red spectra were recorded in the range 4000 to 600 cm^{-1} using a Nicolet 510P Fourier-Transform spectrophotometer. The spectra are reported as peak maxima (ν_{max}) in wavenumbers (cm^{-1}). Spectra from liquid samples were recorded on thin films, and solid samples were mulled with nujol. Infra-red spectroscopy was the least diagnostic of the analytical techniques used, particularly for the identification of crown ether derivatives, but it allowed preliminary assessment of the nature of the product, its purity and the completeness of the reaction. This latter aspect was especially useful during hydrosilylation reactions, which were monitored by noting the decrease and subsequent disappearance of the silicon-hydride, Si-H, functionality which exhibited a strong absorption at ~2100 cm^{-1}. The predominant bands characteristic of both the model and polysiloxanes are in the 1100-1000 cm^{-1} region and are assigned to the asymmetric stretching of the Si-O-Si bond. Characteristic infra-red absorption frequencies for methyl containing siloxanes are summarised in **Table 3.1**.

Wavenumber (cm^{-1})	Group	Assignment
3000-2850	MeSi	C-H stretch
1440-1390	MeSi	C-H asymmetrical bend
1280-1240	MeSi	C-H symmetrical bend
1100-1000	Si-O-Si	Si-O-Si asymmetrical stretch
870-750	MeSi	Me-Si rocking vibration
865-715	MeSi	Si-C stretch
850, 850	Me_3Si	Me-Si rocking vibration
845, 745	Me_3SiO	Si-C stretch
841, 755	Me_2Si	Me-Si rocking vibration

Table 3.1 Infra-red Absorption Frequencies for Methyl Containing Siloxanes

From the analyses listed in the experimental data (Chapter Two), it can be seen that modified siloxanes have characteristic absorption frequencies which occur within the relevant ranges as described above, with the most easily identifiable absorbances as noted in **Table 3.2**.

Wavenumber (cm^{-1})	Group	Assignment
1261-1252	MeSi	C-H symmetrical bend
1049-1020	Si-O-Si	Si-O-Si asymmetrical bend
802	Me_2Si	Me-Si rocking vibration

Table 3.2 Methyl Containing Siloxanes Infra-red Absorbencies Identified in Crown Ether Functionalised Siloxanes.

Infra-red spectra of modified crown ethers and aza-crown ethers have many common features. The strongest absorbances are attributable to the methylene groups and the ether groups. These and some other common features of the spectra of crown ether derivatives and their precursors are shown in **Table 3.3**.

Wavenumber (cm^{-1})	Group	Assignment
3559-3362	O-H	O-H stretch
3329-3196	N-H	N-H stretch
3076-3074	C=C-H	C-H stretch
2951-2814	C-H	C-H stretch
1676-1618	C=C	C=C stretch
1674-1614	C=O	C=O stretch
1389-1352	C-H	C-H stretch
1132-1113	C-O	C-O stretch

Table 3.3 Common Features in Infra-red Spectra of Crown Ether Derivatives and Their Precursors

3.3. Nuclear Magnetic Resonance Spectroscopy

3.3.1. Introduction

A range of n.m.r spectroscopic methods were used. Both ^{1}H and ^{13}C n.m.r. measurements were recorded routinely on each of the organic compounds. In addition model and polysiloxanes were characterised using ^{29}Si n.m.r. The host-guest interactions were studied primarily using COSY ^{1}H n.m.r. spectroscopy. Nuclear magnetic resonance measurements were undertaken using a variety of machines

depending on the nucleus in question and equipment availability. Thus 270, 400 or 500 MHz instruments were used for the following nuclei:-

Spectrometer	Nucleus
JEOL GNM GX FT 270	^{1}H, ^{13}C
JEOL GNM EX FT 400	^{1}H, ^{13}C (DEPT), ^{29}Si, ^{1}H-^{1}H COSY
Bruker FT 500	^{1}H, ^{1}H-^{1}H COSY

Spectra of both the modified crown ethers and of the functionalised siloxanes were recorded in deuterated chloroform ($CDCl_3$) (obtained from Aldrich or Goss). The chemical shift (δ) data described in Chapter Two is expressed in parts per million (ppm) downfield from the internal reference, tetramethylsilane (TMS), except for the siloxanes where TMS was omitted. The COSY analyses on 1:1 crown ether / amino-acid mixtures used deuterium oxide (D_2O) as the solvent due to the insolubility of the amino-acids in most organic solvents.

3.3.2. ^{1}H Nuclear Magnetic Resonance Spectroscopy

Proton n.m.r is a powerful tool for the characterisation of diamagnetic hydrogen-containing organic and inorganic species. ^{1}H has a spin $I = \frac{1}{2}$ and a natural abundance of 99.98% and for this reason offers a ready route to structural information on small samples, typically less than 50 mg in most cases. The ^{1}H spectra of modified crown ether derivatives showed similarities in their chemical shift data and peak splitting patterns, as illustrated in **Table 3.4**.

Chemical Shift Range (δ)	Multiplicity	Assignment
5.85-5.78	m or o	=C<u>H</u>-
5.16-4.92	t	=C<u>H</u>$_2$
3.76-3.50	m	-C<u>H</u>$_2$-O-C<u>H</u>$_2$-
3.17-2.34	t	>N-C<u>H</u>$_2$
2.88-2.69	m or t	-C<u>H</u>$_2$-NR-C<u>H</u>$_2$-
2.85- 2.00	br, s	>N-<u>H</u>
2.20-2.00	t or q	-C<u>H</u>$_2$-CH=

Table 3.4 Chemical Shift Ranges for the Common Structural Features in Modified Crown Ethers

^{1}H n.m.r spectroscopy was used as a diagnostic tool in three ways during the synthesis of functionalised model and polysiloxanes. Firstly it was used to determine the concentration of Si-H groups on the polysiloxane copolymer, secondly as a way of measuring the completeness of the hydrosilylation reaction and the subsequent purity of the crude product, and finally to determine the purity of the product isolated after purification. The Si-H group gives a nuclear magnetic signal in the region 4.9-5.1 ppm and this peak was compared to that of the methyl protons occurring at ~0.0 ppm. Despite the Si-H peak having a similar chemical shift to the vinyl moiety of the modified crown ether (**Table 3.4**), the reaction could be considered complete when there were no peaks present in this region since both residues would be absent in the resulting functionalised siloxane. It has been reported previously[169] that the resonances for the methyl protons in siloxanes are in the range 0.1-0.2 ppm and these studies confirm these findings (**Table 3.5**).

Chemical Shift Range (δ)	Multiplicity	Assignment
1.42-1.31	t or br, s	$OSiCH_2$-$C\underline{H}_2$-
1.00-0.04	t, m, q or br, s	OSi-$C\underline{H}_2$-
0.00	m	$\underline{Me}_3$-SiO, OSi($\underline{Me}$), OSi($\underline{Me}_2$)CH_2-

Table 3.5 ^1H Chemical Shift Ranges for Crown Ether Functionalised Siloxanes

The chemical shift ranges for specific groups shown for both the modified crown ether analogues (Table 3.4.) and the functionalised model and polysiloxanes (Table 3.5.) encompass the minor differences in the final product. These differences are particularly apparent for the aza-crown ethers with the shorter alkenyl chain, since the inductive effect of the nitrogen atom, and possibly the ethereal oxygens, in addition to the silicon atom or the vinyl group will significantly affect the methylene protons in the side-chain.

3.3.3. ^{13}C Nuclear Magnetic Resonance Spectroscopy

Once a satisfactory proton n.m.r. spectrum had been obtained for a product, its carbon spectrum was recorded. ^{13}Carbon is the magnetically active isotope of carbon and has a natural abundance of only 1.1% and a spin I = ½. The carbon nucleus is also much less receptive than the proton nucleus and so ^{13}C .n.m.r. spectra require longer analysis times and larger sample quantities, ~100 mg.

The common structural features of the products could be identified easily in the carbon spectra, including signals from the ethereal carbon atoms, those α-to the nitrogen and those belonging to the side-chain. Their assignments were confirmed by the DEPT technique (distortionless enhancement by polarisation transfer), which allows methyl, methylene and methine carbon atoms to be differentiated. The theory and application of carbon n.m.r spectroscopy is available in appropriate texts[170-173].

The most distinctive chemical shift data common in both the modified crown ethers and the functionalised siloxanes are described in **Table 3.6**. As with the proton data, there are a range of resonances where specific groups may resonate due to small environmental differences.

Chemical Shift Range (δ)	Assignment
173.0-67.9	>$\underline{C}$=O
139.3-132.6	=$\underline{C}$H-
117.2-114.2	=$\underline{C}H_2$
70.9-67.9	-$\underline{C}H_2$-O-$\underline{C}H_2$-
62.6-46.5	-$\underline{C}H_2$-NR-$\underline{C}H_2$-
59.8-31.5	-$\underline{C}H_2$-CH=
56.3-46.7	>N-$\underline{C}H_2$-
29.8-21.0	OSiCH$_2$CH$_2$-$\underline{C}H_2$-
18.4-18.0	OSiCH$_2$-$\underline{C}H_2$-
1.7-0.0	$\underline{Me}_3$-SiO, OSi$\underline{Me}_2$, -OSi($\underline{C}H_2$)$\underline{Me}_2$

Table 3.6 ^{13}C Chemical Shift Ranges for Modified Crown Ether Compounds and Functionalised Siloxanes.

3.3.4. ^{29}Si Nuclear Magnetic Resonance Spectroscopy

^{29}Si is the only magnetically active natural isotope of silicon and has a spin I = ½ and an abundance of 4.7%. The chemical shifts encountered in silicon n.m.r. can cover a very wide range of shifts of around 400 ppm, usually in the range -200 ppm to +200 ppm. However in these studies the typical ^{29}Si resonances recorded in chloroform were typically in a much narrower range, from -22 ppm to +8 ppm, due to the similarity in the environments of the individual silicon atoms. The different silicon species expected in the model and polysiloxanes could usually be distinguished after signal expansion of appropriate segments of the spectrum.

A shiftless relaxation agent, $Cr(acac)_3$, was added to all the samples prior to all ^{29}Si n.m.r. analyses. $Cr(acac)_3$ is a paramagnetic compound which is coordinatively saturated and kinetically inert. It is therefore unlikely to undergo chemical reactions with the sample or selectively relax some atoms in preference to others. The reagent acts a bar-magnet surrounded by hydrophobic groups which make it soluble in organic solvents. The reagent has two main effects. Firstly, it quenches the Nuclear Overhauser Effect (NOE) which could result in the signals being inverted or their intensity reduced. Secondly , it increases the spin-lattice relaxation rates so that all the ^{29}Si nuclei have approximately the same value. Excess of the reagent was avoided since it will increase the relaxation rate to such an extent that the resonances will become too broad to detect.

In these studies three distinct types of silicon nuclei were detected in both the crown ether functionalised model and polysiloxanes. A summary of their chemical shift ranges is given in **Table 3.7**.

Chemical Shift Range (δ)	Assignment
-20.9 to -22.8	$-Me_2-\underline{Si}-O-$
+6.8 to +7.1	$-O-\underline{Si}-(Me_2)CH_2-$
+7.2 to +7.5	$-O-\underline{Si}-Me_3$

Table 3.7 ^{29}Si Chemical Shifts for the Functionalised Siloxane Compounds

In some of the crown ether functionalised polysiloxane copolymers only the intense dimethyl siloxane signals in the range -20.9 to -22.8 ppm could be detected (**Table 3.7**) because of their high concentration relative to both the trimethyl terminal silicons and the silicons on to which the crown ether side-chain is attached.

3.3.5. ^1H-^1H Correlated Spectroscopy (COSY)

Correlated spectroscopy is a relatively recent spectroscopic technique but has become a routine analytical method. It provides one of the most reliable and frequently used two-dimensional experiments. COSY is a method of obtaining greater structural information by showing which nuclei are adjacent to each other. ^1H-^{13}C and ^{13}C-^{13}C techniques are also available, however our studies were concerned with the ^1H-^1H relationship between protons in the receptors, the target amino-acids and 1:1 molar ratios of the two.

It was found that simple one dimensional proton n.m.r. spectroscopy could not satisfactorily elucidate any chemical shift changes which occurred in the mixed samples, because of signals with similar shifts and integrals of similar height which could not be definitely assigned to either the host or the guest. The use of ^{1}H-^{1}H COSY allowed resonances to be correctly assigned and any change in chemical shift to be noted. Further treatment of the COSY data will be described more fully in Chapter Four.

3.4. Instrumental Elemental Analysis

The elemental analyses for C, H and N (where relevant) of the compounds described in Chapter Two were carried out by Mr Alan Carver, School of Chemistry, using a Carlo Erba 1106 Elemental Analyser. A comprehensive account of microanalytical techniques is given by Belcher[174].

3.5. Mass Spectrometry

Mass spectrometry is concerned with the vapour phase formation of ions from neutral compounds and the subsequent examination of their mass:charge ratio and fragmentation patterns. Only very small amounts of material are required for analysis and the results can be extremely valuable in establishing structural data on products, and as an indication of their purity. Mass spectrometry was used in the characterisation of the synthetic hosts and their intermediates where appropriate. Mass spectral data were not determined on siloxane compounds.

Mass spectra were recorded using a VG Analytical 7070E Mass Spectrometer by Mr Chris Cryer, School of Chemistry. Electron Ionisation (E.I.), Chemical Ionisation (C.I.) or Fast Atom Bombardment (F.A.B.) techniques were used as noted in Chapter Two, where the molecular ion, M^+ or $(M+H)^+$, base peak (100%) and other major peaks have been identified.

The E.I. spectra were produced using an ionising potential of 70 eV and this technique was the method of choice since it gives the simplest spectra. However, it is also the technique which causes the most fragmentation and in many cases the molecular ion for the modified crown ethers could not be detected. Under these circumstances the C. I. technique using isobutane was used, and for crown ethers with either one or two side-chain functionalities, the molecular ion was easily detectable, although the spectra as a whole were more complicated due to the inclusion of the reagent gas.

The bis-crown ether receptors required an even softer ionisation technique, since it was found that C.I. was too harsh for the molecular ion to be detected. F.A.B. uses a beam of ions, usually $Xe^{+\bullet}$, to produce fast atoms which are directed at the sample which has been previously mixed with glycerol. The bombardment causes some of the sample to be volatilised and ionised due to the large kinetic energy of the fast atoms. A further discussion of mass spectrometry techniques and their applications is described by Rose and Johnstone[175].

3.6. High Performance Liquid Chromatography

3.6.1. Introduction

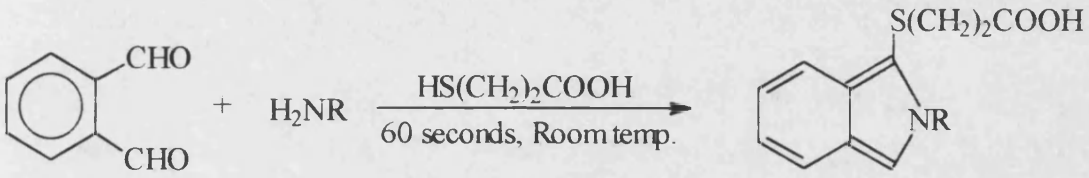
High performance liquid chromatography (HPLC) is a highly sensitive analytical separation technique and can be used for accurate quantitative determinations. It has found widespread use in industry, particularly in pharmaceuticals manufacturing, because of its suitability for separating non-volatile compounds or those which have a low thermal stability. HPLC, like other chromatographic techniques, is based on the basic principle that there is a variation in the rate at which the different components of a mixture will transfer through a stationary phase under the influence of a mobile phase. Thus the separation will be most sensitive to changes in either the mobile phase, the stationary phase, or both. The theory and application of HPLC is well documented in the literature and further references on the subject can be found from this source[176].

3.6.2. Summary of the HPLC Method Employed

This study was primarily concerned with the separation and detection of amino-acids in solution. Their concentration in solution was required before and after extraction with a receptor-functionalised siloxane. The method of detection therefore needed to be sensitive, with very low limits of detection, and good reproducibility. HPLC was deemed to be the ideal technique in this instance. The system used was a Hewlett-Packard HP1090 Liquid Chromatograph equipped with on-line amino-acid derivatisation and a diode array detector. The methodology employed was based on the work by Schuster and Apfel[177].

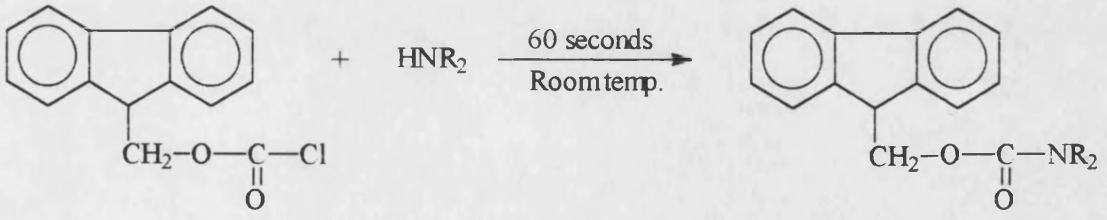
3.6.3. Amino-acid Derivatisation

The derivatisation of compounds under analysis may be necessary in order to optimise the conditions of the system. The analyte may not be separable in the underivatised form or it may be difficult to detect. In the case of amino-acids, derivatisation is required so that they can be determined accurately at low concentrations. The amino-acid derivatives are fluorescent and can be detected down to levels of ng l^{-1}. The method of derivatisation is different for amino-acids depending on whether they contain a primary (1^{o}) or a secondary (2^{o}) amine function. The former are reacted with an equimolar mixture of o-phthalaldehyde (OPA) and 3-mercaptoproprionic acid, as described in **Scheme 8**.



Scheme 8

The latter are derivatised using equimolar 9-fluorenylmethylchloroformate (FMOC) as illustrated in **Scheme 9**.



Scheme 9

HPLC analysis using pre-column derivatisation is very convenient and has found use in the separation of amino-acids from complex and diverse samples such as blood plasma and cheeses[178-179].

3.6.4. Analytical Method

As described earlier the method used was that developed by Schuster and Apfel, although slight modifications were made where necessary to optimise the technique to the operating conditions and the equipment available. The system parameters generally used were :-

Mobile Phase A	Mobile Phase B
20 mmol sodium acetate buffer	20% 100 mmol sodium acetate buffer
	40% acetonitrile
	40% methanol

Column :- Beckmann Ultrasphere ODS 25 cm × 4.6 mm

Flow rate :- 2 ml $minute^{-1}$

Oven temperature :- 40°C

Detector wavelength (λ) :- 338 nm

More information about the precise operating parameters can be found in **Appendix One**.

The development of a methodology which could lead to the determination of the effectiveness of the functionalised polysiloxane as a selective extractant was divided into three distinct but related areas. These were as follows :-

(i) The optimisation of the system using both standard and complex mixtures.

(ii) The development of a calibration curve.

(iii) Analysis of standard amino-acid solutions after extraction experiments.

3.6.4.1. Optimisation of the System.

The target amino-acid was glutamic acid and was one of a number of amino-acid products present in a complex bio-fermentation broth. Before work was done using the broth the system was tested using the complex standard supplied by Hewlett- Packard. The standard sample consisted of 23 primary and secondary amino-acids and was analysed to determine the effectiveness of the separation. From the data the optimum conditions could be applied to the remaining separations.

The system was simplified further by only analysing for primary amino-acids. Although secondary amino-acids were present in both the complex standard and the broth their detection was not necessary for these studies. However their presence affected decisions concerning system parameters such as analysis time and wash cycles, since their complete removal from the system was paramount if reliable and reproducible results were to be gained. Their removal from the analyses was achieved simply by omitting the FMOC derivatisation as described previously.

3.6.4.2 Development of a Calibration Curve

The calibration curve data were obtained by analysing a range of standard glutamic acid solutions having concentrations of 1000, 500, 100, 50 and 10 $ng\mu l^{-1}$. Each of the standards was analysed three times to maximise the precision and accuracy. They were also analysed from the weakest to the highest concentration to minimise any effects of carry-over which would result in an abnormally high response and inaccurate results. Examples of the calibration curves are given **Appendix Two**.

3.6.4.3 Extraction Experiments of Glutamic Acid from Aqueous Solution Using a Crown Ether Functionalised Polysiloxane

Glutamic acid was dissolved in 0.1M HCl in HPLC grade water to give a solution of known concentration. A known volume of this solution was then shaken with an equimolar receptor site equivalent of the crown ether functionalised polysiloxane (FCP). The volume of the standard taken should give an amino-acid concentration which lies within the calibration curve range, ideally at around the 100 ng region. This also kept to a practical minimum the amount of FCP needed. The solution and the FCP were added together and shaken thoroughly for two hours. The mixture was then allowed to stand until the two layers had separated. The aqueous phase was carefully removed. The sample was filtered using a Gelman Acrodisc LC13 PVDF 0.45 μm filter before being analysed as described previously. The polymer used in these studies was BN15C5PS. This was the only material tested since a large amount was needed for sufficient testing, around 15 g in this instance, and it was anticipated that such a simple type of receptor would be an effective extractant (Chapter Four). The conditions for these analytical studies are given in **Table 3.8**.

Molar Equivalent of FCP	Molar Equivalent of Analyte	pH	pH adjusted using
1	1	0.8	None[a]
1	1	7.2	KOH
1	1	10.8	KOH
10	1	7.08	KOH
10	1	8.0	TEAH[b]
10	1	3.4	None[c]

[a] pH of solution in 0.1M HCl [b] tetraethylammonium hydroxide

[c] sample dissolved in HPLC grade water only

Table 3.8 Conditions used for the Extraction Studies

The extractions were attempted at different pHs as the structures of amino-acids are pH dependant as they are amphoteric and can exist as zwitterions. The zwitterionic forms of GABA and glutamic acid and the pK_a of the respective protons are shown in Chapter 4 **(Figures 26 and 27)**.

The inclusion of KOH and TEAH in the analyte did not affect the results significantly although fractionally longer retention times were noted when compared to data where these reagents were absent. TEAH was an alternative base, used in place of KOH, in order to avoid the possibility of any K^+ interference with the crown.

The extractions were made at pH values where the glutamic acid should have been as its protonated amine zwitterionic form, and so is most likely to undergo hydrogen bonding with the crown ether receptor. However no extraction from aqueous solution was detected, with the standard solution having the same amino-acid concentration both before and after mixing with the FCP. Thus the host-guest interactions are not sufficiently strong to bind the amino-acid and permit extraction in the presence of a large excess of water.

Chapter Four

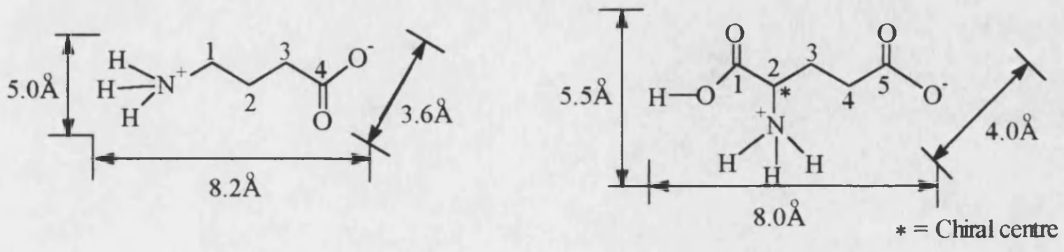
Results and Discussion

4.1. Introduction

The preparation of pharmaceutical compounds and their intermediates using fermentation processes produces a wide variety of by-products which usually depend on the initial concentration and quality of the fermentation ingredients and on the conditions employed in the fermentation process. The formation of these by-products may interfere with subsequent synthetic manipulations, or analytical determinations on the target compound. The industrial collaborator involved in the project was interested in the specific fermentation by-products, 4-aminobutan-1-oic acid (GABA) and glutamic acid, which were interferants in post-fermentation assays being carried out on the fermentation broths. The removal or masking of these contaminants was of immediate interest, and in a broader context, the general theme of the recognition and complexation of organic acids and amino-acids by crown ethers and their derivatives formed a major component of this research project.

4.2. Structural Features of 4-Aminobutan-1-oic Acid (GABA) and Glutamic Acid

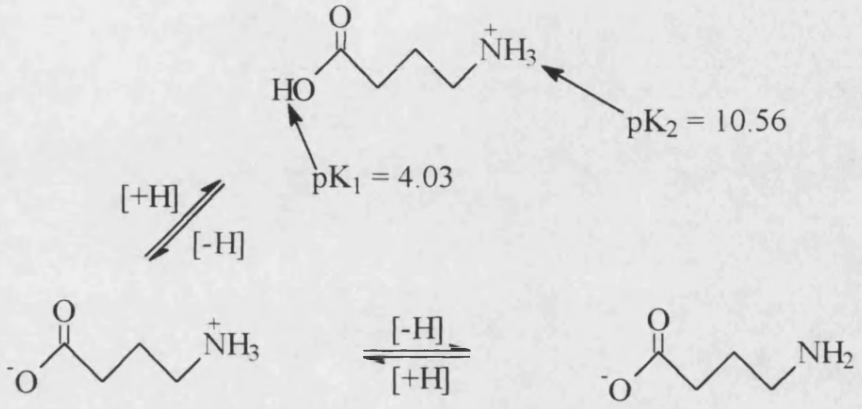
GABA (4-aminobutan-1-oic acid) and glutamic acid (2-aminopropan-1,5-dioic acid) are both amino-acids and their approximate dimensions, based on unit cell data taken from solid-state structural analyses of the compounds, are shown in **Figure 25**.



GABA Glutamic acid

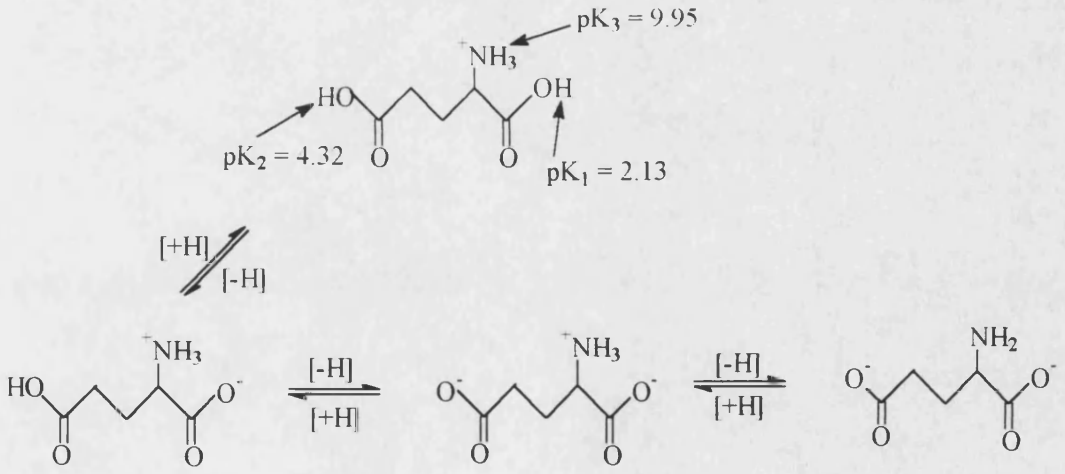
Figure 25

Amino-acids exist as zwitter-ions in neutral aqueous solution and their structures are affected by changes of pH. The proton pKa's, pK_1, pK_2 and pK_3 where appropriate, for GABA and for glutamic acid are shown in **Figures 26** and **27** respectively.



The protonation / deprotonation sequence and pK_1 and pK_2 values for the labile protons in GABA

Figure 26



The protonation / deprotonation sequence and pK_1, pK_2 and pK_3 values for the labile protons in Glutamic Acid

Figure 27

The degree of protonation of the amino-acid guest, whether it is a fully protonated cation, a zwitter-ion, or a deprotonated anion, will affect host-guest complexation as well as the strength of any intermolecular non-covalent interactions between the host and guest. Thus pH variation offers a means of controlling both the complexation and decomplexation reactions.

The solid-state structure of GABA was first reported by Steward et al[180] (**Figure 28**). They confirmed the presence of the zwitter-ion by identifying three hydrogen atoms bonded to each tetrahedral nitrogen atom. Intermolecular hydrogen bonds between each of the ammonium hydrogens and oxygen atoms in neighbouring GABA molecules resulted in a cross-linked arrangement of zwitter-ions in the lattice. The molecule was found to adopt a gauche conformation about the C(2)-C(3) axis, in contrast to the trans configuration found in GABA·HCl (4-aminobutanoic acid hydrochloride)[181-182].

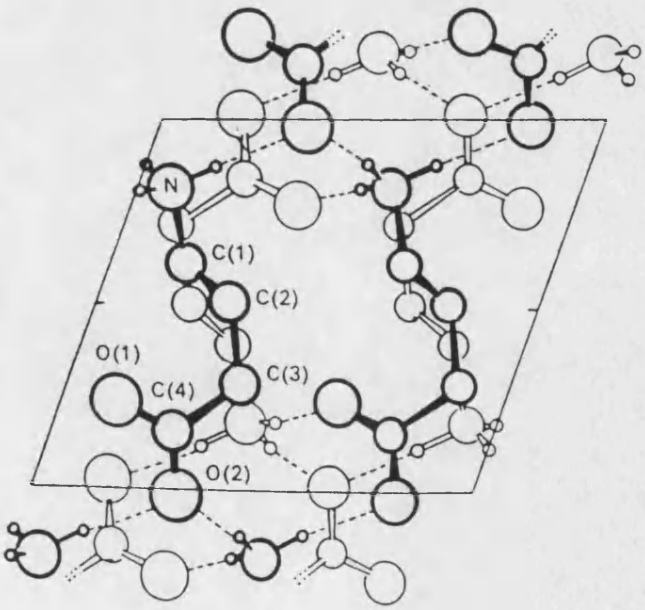


Figure 28

Later studies by Craven and Weber[183], on the charge density in crystals of GABA cooled to 122K, showed that the gauche conformation was a result of an intramolecular hydrogen bridge. A proton on the C(2) carbon (**Figure 28**) has a significant positive charge (+0.11e) induced as the molecule folds into the gauche conformation. This proton is within short intramolecular distance of the two electronegative N (-0.23e) and O (-0.15e) atoms and the resulting H bridge, N---H---O, contributes to the stabilisation of the observed conformation. Molecular orbital calculations[184] on isolated molecules indicate that the trans conformation is of lower energy than the gauche form by some 33.4 kJ mol^{-1}. These results raise the possibility that more than one conformer of GABA may exist in solution in polar solvents.

Glutamic acid has two distinct polymorphs designated α and β. The α-form occurs when crystals are deposited relatively quickly from solution and it is slowly converted to the more stable β-conformation on standing in aqueous solution. The β-form is also obtained when glutamic acid crystals are grown slowly from water[185]. In both the α- and β- solid states the molecules are in a zwitter-ionic form and exhibit strong OH---O intermolecular hydrogen bonds resulting in chain formation. Further intermolecular hydrogen bonding involving $^{+}$NH---O links accounts for the final three-dimensional networks found in the lattices for both polymorphs. In the α-structure the C(1) carbonyl oxygen forms a bifurcated hydrogen bond with a C(5) carboxyl hydrogen and a NH_3^+ group, and the other carboxylate oxygen forms a hydrogen bond to a NH_3^+ residue in an adjacent molecule (**Figure 29**).

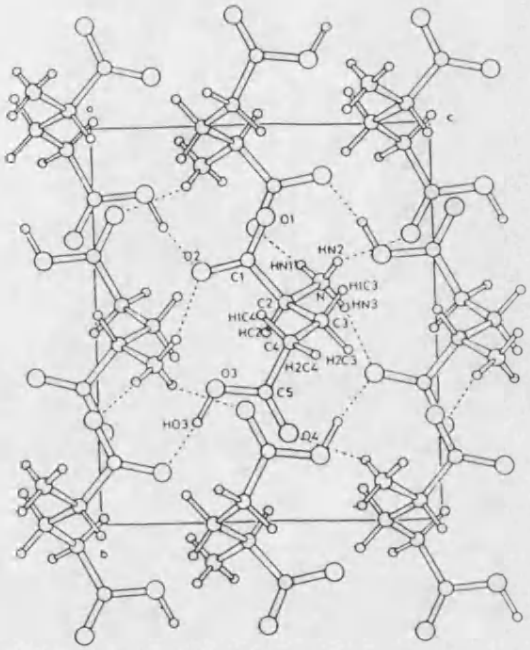


Figure 29

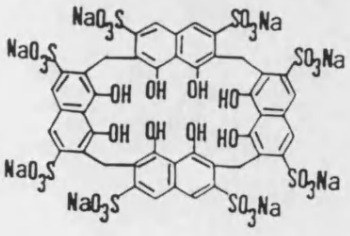
The β-conformer differs from the α-form in that the C(1) carbonyl oxygen is hydrogen bonded to two NH_3^+ residues, with the other oxygen on C(1) linking to a C(5) OH group of a neighbouring molecule. In the α-form the conformation around the C(2)-C(3) and the C(3)-C(4) bonds are both gauche whereas in the β-form they adopt the trans-gauche orientation.

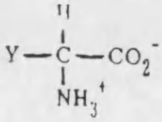
Both GABA and glutamic acid, like all amino-acids, exhibit extensive hydrogen bonding and are therefore potential guests for appropriately designed polar receptors such as crown ethers. As amino-acids are also effective ligands towards metal ions the prospect of using metal ion-containing species as an alternative type of host[186] is an interesting possibility. Takenaka et al[187] reported the crystal structure of a copper(II) bis-GABA complex $[Cu(NH_2CH_2CH_2CH_2COO)_2]_n$.

This compound exhibits an infinite one-dimensional chain structure in which the copper(II) atoms are chelated by two trans carboxylate groups from two GABA ligands. The remaining co-ordination sites on the metal are filled by nitrogen donation from the amine moiety of a third GABA molecule and water, resulting in a distorted octahedron about the copper(II) atom. The conformation around the C(2)-C(3) axis is described as "almost gauche", being very similar to free GABA and showing that the intramolecular hydrogen bridge remains largely undisturbed on binding to copper.

4.3. The Recognition of Amino-acids and Their Derivatives by Synthetic Host Compounds

The complexation of amino-acids and their derivatives, through complementary intermolecular hydrogen-bonding with a suitable receptor, would be expected to occur primarily through either the protonated amine group, the carboxylic acid residue, or through a combination of both these moieties. Interestingly n.m.r. studies on the complexation of amino-acids by cyclotetrachromotropylene (**Figure 30**) in aqueous solutions also revealed the importance of CH-π and π-π interactions[188]. Each guest molecule is held within the host cavity of this receptor, which results in complementarity between host and guest areas of both polar and non-polar character.





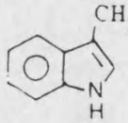
	Y		Y
2	H	8	CH_2CONH_2
3	CH_3	9	CH_2CO_2H
4	$CH(CH_3)_2$	10	$(CH_2)_2CO_2H$
5	$CH(CH_3)CH_2CH_3$	11	$(CH_2)_4NH_2$
6	$CH(OH)CH_3$	12	$CH_2C_6H_5$
7	$(CH_2)_2SCH_3$	13	

Figure 30

Of more relevance to this study are other reports on the use of crown ether receptors for the complexation of GABA derivatives and of amino-acids. Vaccher et al[189] have reported on the enantiomeric resolution of GABA derivatives using a chiral crown ether stationary phase in an HPLC system (**Figure 31**). Whilst no host-guest complex was isolated, the principles involved in the resolution process are of relevance to the complexation of GABA itself.

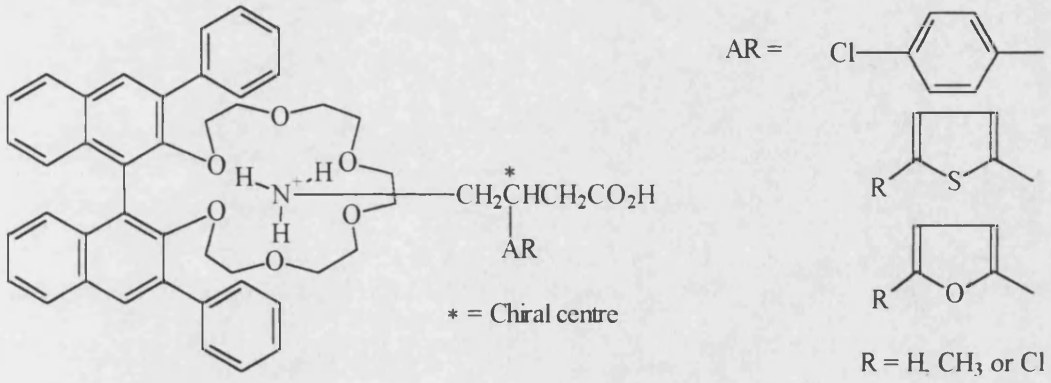


Figure 31

Studies on the molecular recognition of amino-acids are more numerous. Tsukube and co-workers[190] used a bipyridine functionalised monoaza-18-crown-6 receptor to complex histadine, lysine and glycine amino-acid esters. The bipyridine lariat was used to complex a lanthanide complex, $Dy(fod)_3$, which in turn co-ordinated to the ester carbonyl oxygen of the guest, whilst the crown ether bound the protonated amine residue (**Figure 32**).

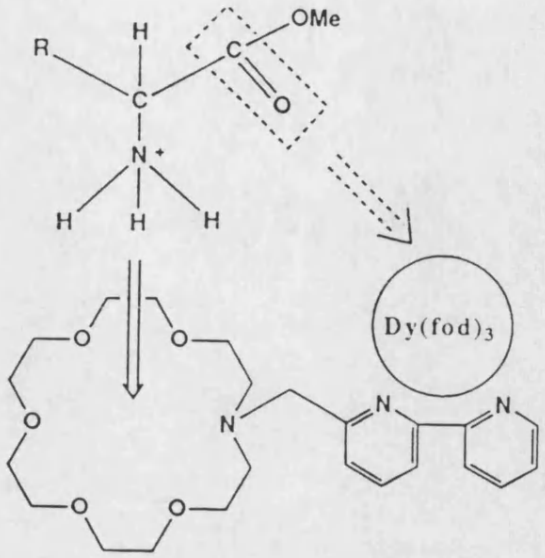


Figure 32

Initially the binding behaviour of the $Dy(fod)_3$ complexes towards histamine, lysine and glycine amino-acid esters was investigated using ^{13}C n.m.r spectroscopy. The results showed that the strength of the interaction, as revealed by induced chemical shifts of guest carbon atoms, was larger when the bipyridyl unit was attached at its 2-position to the crown ether, as shown in **Figure 32**, than when linked via the 3-position. Transport of amino-acid ester salts from one aqueous phase to another across a dichloromethane membrane, in which the crown ether / $Dy(fod)_3$ was dissolved, was also demonstrated. The effect on the rate of transport of a specific amino-acid ester on replacing Dy with other lanthanides was also determined, with the rate increasing in the series $Dy(fod)_3 > Yb(fod)_3 > Eu(fod)_3 >> Pr(fod)_3$.

Another significant study involving the recognition of amino-acid derivatives by crown ethers containing hydrophobic appendages was reported by Kawabata and Shinkai[191]. 4'-(Cholesteryloxycarbonyl)benzo-18-crown-6 was employed as a chiral receptor which traps the $-CH_2R$ residue of phenylalanine, alanine, tryptophan and valine amino-acid esters in the cholesterol stacks after the polar $^+NH_3$ group has been bound to the crown ether ring. In aqueous media the crown ether lies at the water's surface with the cholesterol unit projecting away from the aqueous phase. After compression the resulting monolayer system responds to amino-acid derivatives as shown in **Figure 33**, and it can thus be used as a novel sensor.

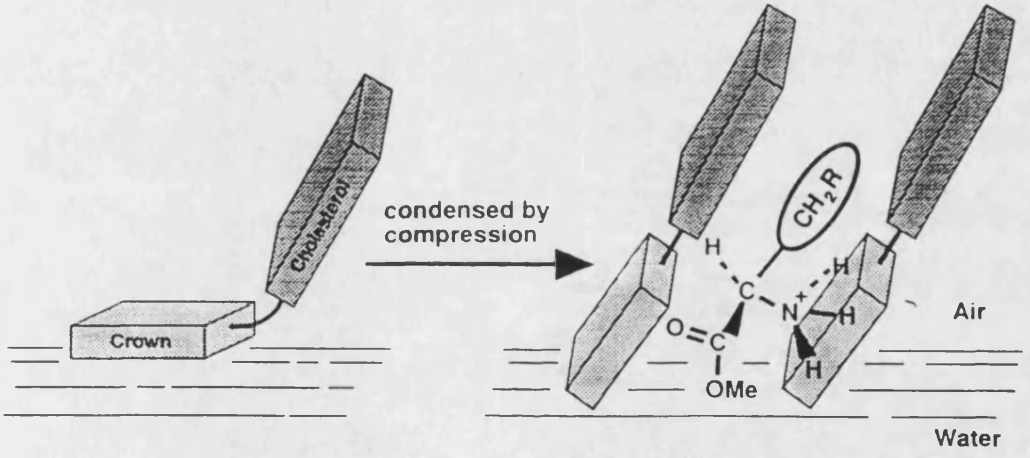


Figure 33

Very recently (1996), Metzger et al[192] reported the molecular recognition and phase transfer of underivatised amino-acids by a foldable artificial host based on a pyrimido-pyrimidine lariat functionalised triaza-18-crown-6 (**Figure 34**). They noted that, in general, in order to transfer selectively an amino-acid from an aqueous environment to a low polarity organic phase, the solvation shells around the highly hydrophilic and heavily hydrated carboxylate and ammonium moieties must be

replaced by dedicated ligands which specifically interact with these epitopes and compensate for the energetic cost of desolvation. The functionalised triaza-18-crown-6 host noted above was shown to be an effective extraction agent for five zwitter-ionic amino-acids in the order Phe > Leu > Trp > Gly >> Ser. The extractions, as expected, were dependant on the solution pH with the optimum extraction occurring at around pH 9.

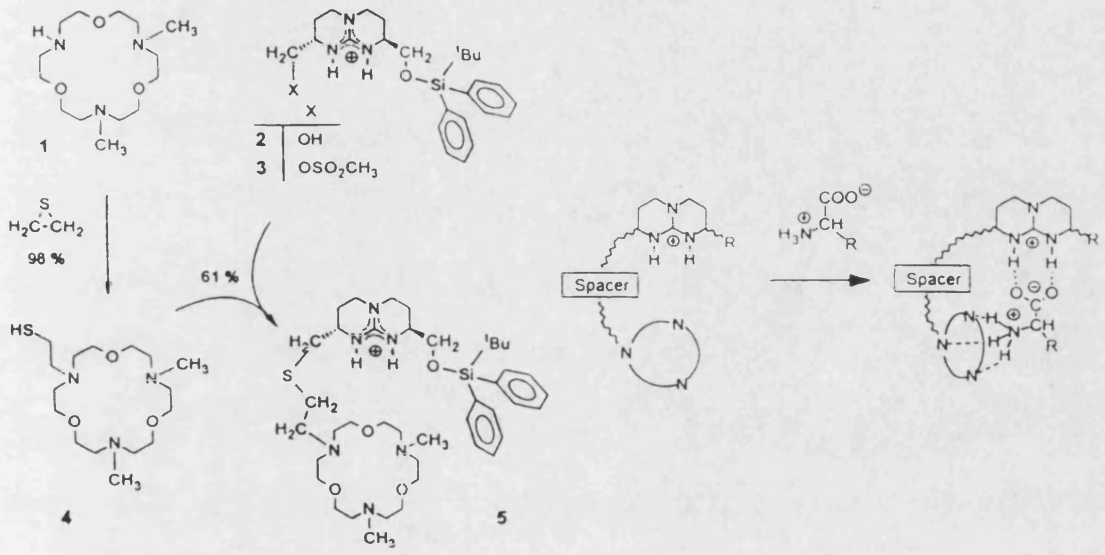


Figure 34

This study by Metzger, together with that carried out by Flack et al[61] on diaza-18-crown-6-bis(amidopyridine) receptors referred to earlier (**Chapter 1** ; **Figure 19**), show the importance of having a residue on the receptor which can take part in hydrogen bonding with carboxylic acid or carboxylate residues of organic- and amino-acids. The investigation by Flack et al also highlighted the importance of host cavity size and complementarity in orientation of the host-guest hydrogen bonding

sites when attempting to extract organic diacids from aqueous solution using host-guest concepts. Thus maleic acid, the cis isomer of butanedioic acid, was extracted into chloroform by the diaza-crown ether receptor but fumaric acid (the trans isomer) was not. Maleic acid has the correct dimensions and disposition of functional groups to fit into the receptor cavity whereas fumaric acid, having greater separation of the two binding functionalities, does not fit into the cavity whilst presenting the carboxylate residues to receptor sites.

4.4. The Development of Crown Ether Derivatives to act as Receptors for GABA and Glutamic Acid

In view of previous work in these laboratories on amine cation - crown ether interactions[113], and the literature reports referred to above, crown ethers and particularly their aza-analogues were the first and simplest receptor species to be investigated in this programme. The main unit upon which the receptors were constructed was monoaza-18-crown-6, although monoaza-5-crown-5, 12-crown-4 and 15-crown-5 hosts were also synthesised and their complexing behaviour explored. Macrocycles containing only oxygen donors were made primarily to gain experience in crown ether synthesis and to allow comparison of their host properties with those of their nitrogen containing analogues. In addition, as oxa-crown ethers do not contain a strongly basic centre they are not readily protonated, thus eliminating the possibility of proton transfer reactions occurring from the protonated guest to the host.

Since the dimensions and conformations of GABA and glutamic acid are available from literature structural data, the approximate dimensions of potential crown ether hosts could be derived by consideration of standard covalent bond lengths and angles (**Table 4.1**). The results of these simple calculations are considered later.

Bond	Length(Å)	Angle
C_{sp3}-C_{sp3}-C_{sp3}	1.54	109° 28'
$>C_{sp2}=C_{sp2}<$	1.34	120°
C_{sp3}-H	1.09	109° 28'
$>C_{sp2}=O$	1.22	120°
C_{sp3}-O-C_{sp3}	1.43	104.5°
C_{sp3}-N(C_{sp3})-C_{sp3}	1.47	107°

Table 4.1. Average Bond Lengths and Angles Used to Determine Spacer-Chain Length and Host Sizes.

In order to attach a suitable crown ether to a hydride-containing polysiloxane, a non-active 1-alkenyl functionality was required on the host. The length of the alkenyl side-chain, and hence the separation between the crown ether and the siloxane polymer chain could affect host-guest interactions through steric effects and changes in chemical environment. The approximate separation expected between the nitrogen atom of the crown ether and the polymer chain was calculated as a function of a linear spacer-chain using the dimensions and measurements from **Table 4.1** and is given in **Table 4.2**.

Number of Carbon Atoms	Length (Å)
3	4
4	6
6	8
8	10

Table 4.2. Alkenyl Side-Chain Length Expected For 1-Alkenyl Functionalised Crown Ethers.

Calculations on the active host sites indicated that the monoaza-18-crown-6 receptor could bind to either GABA or glutamic acid via hydrogen bonds involving either the ammonium residue, or the carboxylate group, or both in combination with two crown ethers. The mode of interaction if only one functional group is involved is likely to be similar for both guest compounds. However, if both the ammonium and carboxylate residues of the amino-acids are simultaneously involved in binding, for example by using a bis(crown ether) receptor, then the different structural features of GABA compared with glutamic acid may invoke different docking arrangements with the host.

The simultaneous complexation of both the protonated amine and the carboxylate functions of GABA can only occur when the guest is capped at both ends by a receptor. An inter-receptor site distance of approximately 8Å is required for this mode of interaction (cf **Figure 25**). Inclusion of water may aid hydrogen bonding (**Figure 35**) and complexes involving ROH-crown ether interactions, where the incorporation of water within the coronand frequently occurs, leads to the creation of a net-work of H-bonds[193] which enhance hydrogen-bonding of the guest.

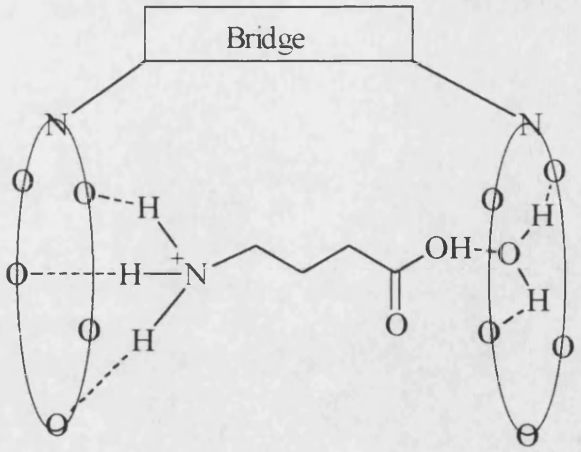
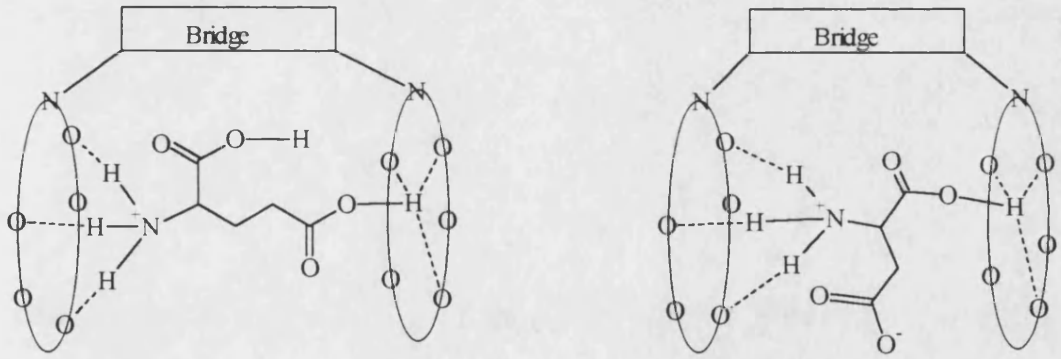


Figure 35

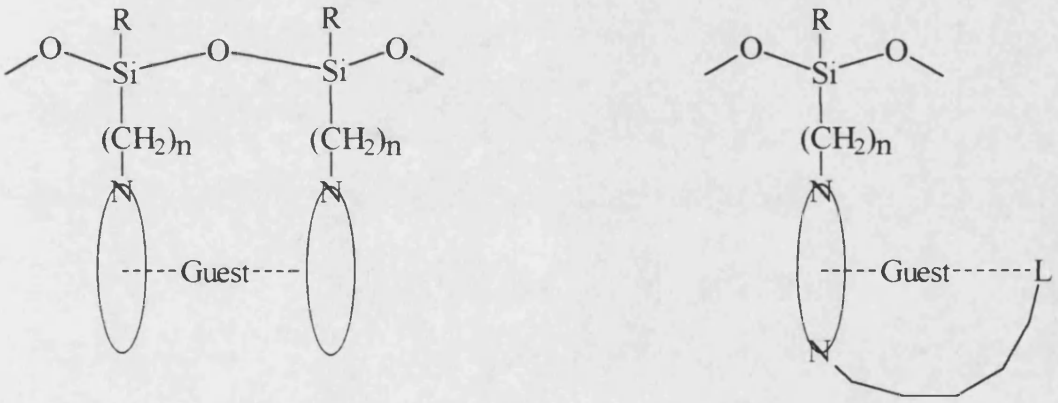
Complexation of glutamic acid could occur in two ways, these being a) "lengthways" and b)"widthways" (**Figure 36 a** and **b**).



a **b**

Figure 36

Lengthways complexation of glutamic acid also requires an inter-receptor separation of approximately 8Å, which would permit intermolecular hydrogen bonds between the bis(monoaza-crown ether) rings and the 5-carboxylate group and the ammonium cation of the guest. Alternatively, binding of the two crown ether rings with the ammonium moiety and the 1-carboxylate group of the guest would result in "widthways" complexation, with the ideal crown ether receptor spacing being around 6Å. The bridge could be completely rigid or flexible. A flexible bridge could adjust to the requirements of the host. This would occur in a fluid siloxane matrix (**Figure 37a**), where individual receptors could organise themselves readily. A slightly less flexible bridge would result from a siloxane linked lariat crown ether system (**Figure 37b**).



(a) **(b)**

Figure 37

Complexation in all cases would be pH dependent. The calculated non-strained inter-receptor site dimensions expected for the bis(monoaza-crown ether) receptors and for the lariat functionalised monoaza-18-crown-6 (pages xvi-xviii) are shown in **Table 4.3**.

Receptor	Minimum Cleft Size (Å)
BisN18C6Sub	10
BisN18C6Fum	5
BisN18C6Ita	5
BisN18C6Iso	5
BisN18C6Iso(Red)	5
BisN18C6Muc	6
HOLN18C6	6

Table 4.3. Approximate Minimum Cleft Sizes Predicted For the Bis- and Lariat-Functionalised Aza-Crown Ether Receptors.

4.5. Studies of Host-Guest Complexation

The stoichiometry and the strength of the interaction between a host / guest pair in a complexation reaction can, in general, be investigated using a variety of techniques including titrimetry, potentiometry, n.m.r spectroscopy and fast atom bombardment mass spectroscopy[194-198].

A commonly used method of determining both the host-guest stoichiometry, and the approximate strength of the interaction, as well as the sites involved in complexation, involves n.m.r. techniques. The changes in the proton chemical shift for the host-guest combination in comparison with the free host and the free guest are due to intermolecular interactions and may reveal which sites are involved in significant host-guest interactions, with the size of the shift giving an indication as to the relative strength of the interactions. This spectroscopic method has been used to good effect by Vögtle and Hoss who examined the interaction between the

macrocycle, tetrahydroxycyclophane, and varying concentrations of the guest, piperazine, in order to determine the parameters noted above (**Figure 37**)[199].

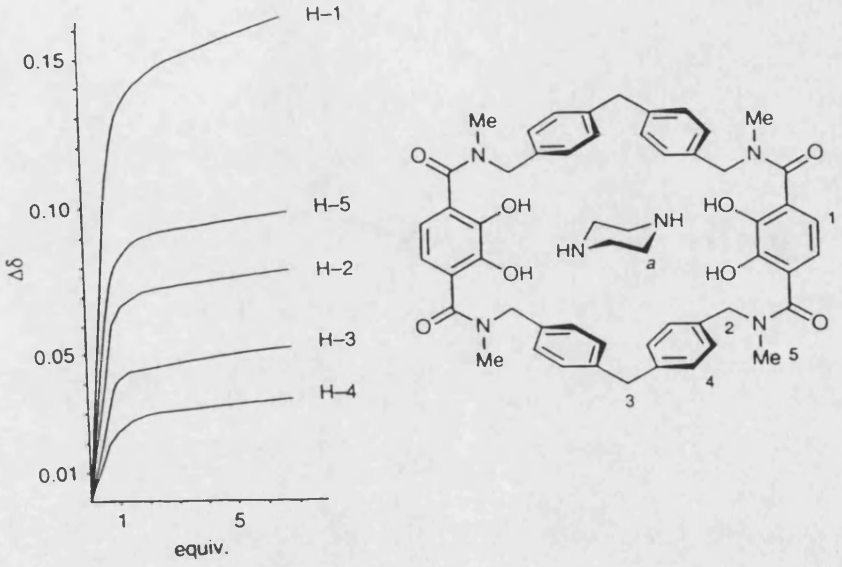


Figure 37

Using the graph depicted in **Figure 37** as an illustration, the stoichiometry of the host-guest interaction is indicated by the point at which the chemical shift changes ($\Delta\delta$) for the host cease to change significantly on further increase in concentration of the guest. In the example shown a 1:1 complex is formed. The strength of the host-guest interaction, the enthalpy of formation (ΔH), is given by the slope of the graph at its steepest point, with a steeper slope indicating a stronger interaction.

Early investigations in this project centred on the use of simple organic acids (valeric acid, *mono*-methyl glutarate and glutaric acid) as model guest compounds in 1:1 combinations with unfunctionalised monoaza-15-crown-5 in order to assess the effectiveness of the n.m.r technique. It was found that as many of the resonances for both the host and the guest species in the spectrum of the 1:1 complex had similar peak heights, any changes in the chemical shift of specific groups on either compound could not be assigned with any confidence when using one-dimensional n.m.r. spectroscopy. Therefore H-H COSY n.m.r spectroscopy was employed since it allowed the precise assignment of all the protons in the spectrum.

The proton chemical shift data for the respective individual host and guest components and for the 1:1 mixed solutions are tabulated in **Tables 4.1** to **4.38**. The change in the chemical shift ($\Delta\delta$) of a given proton for an uncomplexed species, relative to a complexed species, is also given. A change in the proton chemical shift to a higher field position than in the uncomplexed substrate is given as negative (-). Conversely a chemical shift change to a lower field, with respect to the original substrate, is given as positive (+).

The pH of the sample solution has also been noted since this would determine the form of the guest species present in solution (**Figures 26** and **27**). As the pH of the fermentation medium of interest is in the region of pH 7, the most appropriate pH range for the study of both GABA and glutamic acid was between pH 4 and pH 9, under which conditions the amino-acid is in its zwitter ion. Most of the mixed samples had a pH of around 7. Samples were also studied at pH >10 and at pH 1 in order to determine whether complexation or decomplexation of GABA or glutamic acid could be encouraged under these conditions.

Four types of crown ether receptor of increasing structural complexity were prepared, and their complexing ability assessed towards the target organic species. These were :-

(a) Unfunctionalised mono- and diaza-crown ethers
(b) aza- and oxa-crown ethers with a guest-inactive substituent useful for polymer attachment.
(c) lariat aza-crown ethers with active, polar substituents which could provide an additional hydrogen bonding interaction with the guest.
(d) bis(monoaza-crown ethers) containing C_4-C_8 alkenyl or aryl bridges designed to simultaneously bind both the ammonium and carboxylate residues of the guest.

4.6. a) Unfunctionalised Mono- and Diaza-Crown Ethers

4.6.1. Synthesis of Unfunctionalised Crown Ethers

Three unfunctionalised crown ethers were synthesised as starting materials during the course of this study, these being monoaza-15-crown-5, monoaza-18-crown-6 and diaza-18-crown-6. Unfunctionalised oxygen crown ethers were not prepared because they could not easily be functionalised after cyclisation.

Monoaza-crown ethers were prepared following the method published by Maeda et al[94]. In our hands the percentage yields of monoaza-15-crown-5 and monoaza-18-crown-6 were 55% and 71% respectively. These are slight improvements on the literature yields of 37% and 61% for the 15- and 18-membered macrocycles respectively. Higher yields were obtained when oligoethylene ditosylates rather than oligoethylene dichlorides were used in the cyclisation stage.

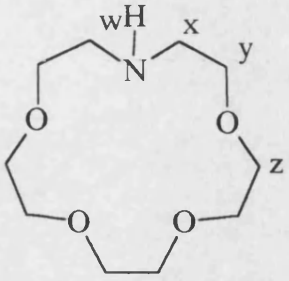
Using twice the molar equivalent of diethanolamine was also found to improve the yield. The synthesis of diaza-18-crown-6 followed a slight modification of the method used by Kulstad and Malmsten[164] and an overall yield of 10% was obtained, which is very similar to that reported in the literature. Unlike the monoaza-crown ethers, the use of oligoethylene ditosylates, although convenient, decreased the yield of product compared to the diiodopolyether which was reported as giving the optimum yield.

4.6.2. Interaction Studies Between Unfunctionalised Crown Ethers and Organic Acids

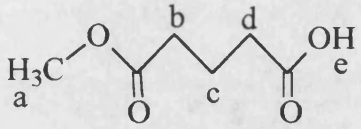
Preliminary n.m.r. experiments involved 1:1 equimolar mixture of monoaza-15-crown-5 and valeric acid (Val), *mono*-methyl glutarate (MMG) and glutaric acid (Glu) (**Tables 4.1** to **4.4**). The crown ether concentration in these and in other samples in general was approximately 0.5 molar and all measurements were taken at ambient temperature in the solvents noted in the individual tables. The chemical shifts are given relative to the solvent which was used as an internal reference. The chemical shift of $CDCl_3$ was 7.27ppm and the chemical shift of D_2O was 4.65ppm. Standardising the solvent chemical shifts allowed the data obtained using different n.m.r. instruments to be compared and contrasted.

The nature of the solvent is of prime importance in host-guest interactions, and Spencer and co-workers[200] reported that the formation of a host-guest complex was favoured in solvents which weakly solvate the uncomplexed components or alternatively strongly solvate the resultant complex. They also note that in hydrogen bonding solvents the solvation of the crown ether is due primarily to hydrogen bonding of the solvent to the crown ether. Although some samples in this study were

dissolved in $CDCl_3$, D_2O was more appropriate as a solvent since the ultimate aim of the project was to extract specific amino-acids from aqueous solutions.



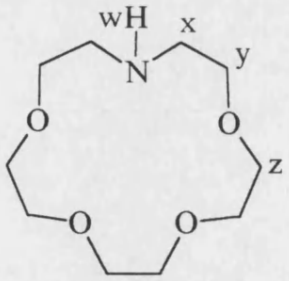
N15C5

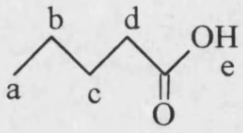


MMG

Proton	δ($CDCl_3$)	δ(N15C5/MMG)	Δδ(N15C5/MMG)
w	2.93	----	----
x	2.75	3.08	+0.33
y	3.63	3.65	+0.02
z	3.63	3.65	+0.02
a	3.69	3.75	+0.06
b	2.43	2.37	-0.06
c	1.96	1.89	-0.07
d	2.43	2.26	-0.17
e	11.01	8.18	-2.83

Table 4.1 ^1H N. M. R. Chemical Shift Data for 1:1 N15C5 / MMG

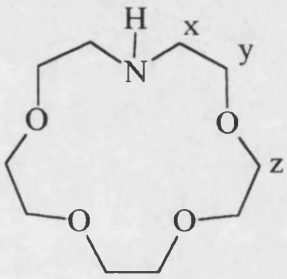


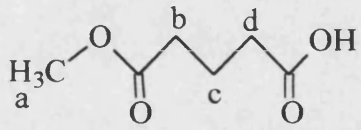


N15C5 **Val**

Proton	$\delta(CDCl_3)$	δ(N15C5/Val)	Δδ(N15C5/Val)
w	2.93	----	----
x	2.75	2.99	+0.24
y	3.63	3.65	+0.02
z	3.63	3.72	+0.09
a	0.93	0.92	-0.01
b	1.38	1.36	-0.02
c	1.63	1.58	-0.05
d	2.36	2.23	-0.13
e	11.92	7.56	-4.36

Table 4.2 ^{1}H N. M. R. Chemical Shift Data for 1:1 N15C5 / Val

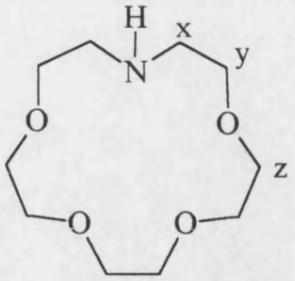


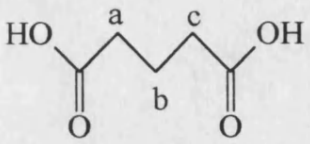


N15C5 **MMG**

Proton	$\delta(D_2O)$	δ(N15C5/MMG)	Δδ(N15C5/MMG)
x	2.71	3.27	+0.56
y	3.64	3.77	+0.13
z	3.64	3.64	0.00
a	3.61	3.64	+0.03
b	2.35	2.35	0.00
c	1.81	1.79	-0.02
d	2.35	2.15	-0.20

Table 4.3 ^{1}H N. M. R. Chemical Shift Data for 1:1 N15C5 / MMG





N15C5 **Glu**

Proton	$\delta(D_2O)$	δ(N15C5/Glu)	$\Delta\delta$(N15C5/Glu)
x	2.71	3.20	+0.49
y	3.64	3.71	+0.07
z	3.64	3.58	-0.06
a	2.20	2.30	+0.10
b	1.71	1.74	+0.03
c	2.20	2.30	+0.10

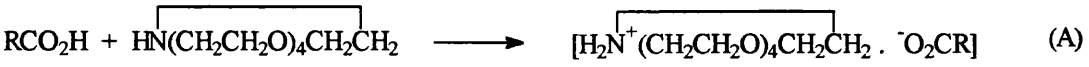
Table 4.4 ^{1}H N. M. R. Chemical Shift Data for 1:1 N15C5 / Glu

From the data above it can be seen that the methylene protons of the monoaza-15-crown-5 α- to the nitrogen exhibit chemical shift changes of +0.33 and +0.24 ppm respectively on mixing the monoaza-15-crown-5 with equimolar amounts of MMG or Val in $CDCl_3$. The same protons showed shifts of +0.56 and +0.49 respectively when the monoaza-15-crown-5 was mixed with MMG and Glu in D_2O. These differences for monoaza-15-crown-5 / MMG combinations may be due to the different solvent effects of $CDCl_3$ compared to D_2O, with D_2O able to become involved in hydrogen bonding between the host-guest pair, so having a greater effect on the chemical shift changes.

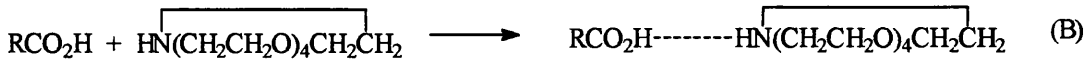
The hydroxyl proton resonances of both MMG and Val are clearly defined when the spectra are obtained in $CDCl_3$ (**Tables 4.1** and **4.2**), and they exhibit very

large changes in chemical shifts in the 1:1 mixtures in comparison with those recorded for the individual compounds (-2.83 ppm and -4.36 ppm respectively). The methylene protons α- to the acid group of all the guests show the next largest changes in chemical shifts of between 0.10 ppm and 0.20 ppm. However, the amine proton of the aza-crown ether was only detected in the unmixed sample. There may be two reasons for this. Firstly, the absence of the resonance may indicate that the host-guest interaction is occurring via the amine moiety of the monoaza-15-crown-5 and some part, most likely the acid residue, of the guest and that the resulting hydrogen bonding renders the amine proton undetectable. Secondly, and more simply, the resonance may have been masked by the increased number of peaks in the spectrum. It should be noted that in general the chemical shift of NH protons, like those of OH protons, are variable as conditions change because the extent to which the hydrogen atoms are involved in hydrogen bonding is both unpredictable and concentration dependent. The signal may also be very broad from quadrupole relaxation of the nitrogen atom, thus making the resonance difficult to detect.

By comparing the results of chemical shift changes of the hydroxyl proton and methylene protons α- to the acidic group in all the guests studied (**Tables 4.1** to **4.4**), it seems likely that the main host-guest interactions involve the carboxylate group of the organic acid and the nitrogen centre of the monoaza-crown ether, with the remainder of both molecules appearing to take little part in the interaction. This is consistent with complete proton transfer from the acid to the basic nitrogen in the macrocycle (A), as opposed to the alternative mode of interaction which involves hydrogen bonding between the two neutral species (B).



(A)



(B)

4.7. b) Oxa-, Aza- and Diaza-Crown Ethers Containing a Guest-Inactive Substituent

4.7.1. Synthesis of Non-Active Mono- and Difunctional Crown Ethers

The hexenyl functionalised oxygen crown ethers, H12C4 and H15C5, were prepared following the method of Abed-Ali et al[113] using lithium and sodium ions respectively as the templating species. Both compounds were prepared using octenyl-1,2-diol, which is readily commercially available, with a molar equivalent of the appropriate oligoethylene glycol ditosylate to form the ring (**Scheme 4, Chapter One**). The crude oxa-crown ethers were distilled, using a kugelrohr apparatus, to give the functionalised oxa-crown ether products as clear, colourless oils with yields in the region of 30%, which are similar to those previously reported.

The attachment of a n-alkenyl functionality to the nitrogen centre of a mono- or diaza crown ether was achieved by a simple nucleophilic substitution reaction using an appropriate n-bromoalkene as described by Abed-Ali et al[113]. In this way 1-alkenyl substituents with 3, 4 and 6 carbon atoms were introduced onto monoaza-15-crown-5 and -18-crown-6. Additionally, an octenyl side-chain was attached onto monoaza-18-crown-6, with 4-bromobut-1-ene and 6-bromohex-1-ene used as the nucleophiles to difunctionalise the diaza-crown ethers. All of these compounds were purified by distillation under reduced pressure to give the products as clear colourless or pale yellow oils, obtained with yields ranging from 37% to 74%. The 95% yield

reported in the literature for the alkenyl-functionalised monoaza-18-crown-6 was not achieved in these studies.

4.7.2. Attachment of Functionalised Crown Ether Analogues to Model Trisiloxanes and to Polysiloxane Copolymers

Two short chain siloxanes, 1,1,1,3,3,5,5- and 1,1,1,3,5,5,5-heptamethyl trisiloxane, having a central- or a terminal-hydride functionality respectively, were used as model compounds for the preparation of functionalised crown ethers. Three trisiloxanes containing an oxa- or aza-crown ether, HN15C5MST, BN18C6MST and H15C5MSM (**Figure 36**), were prepared via a platinum catalysed hydrosilylation procedure.

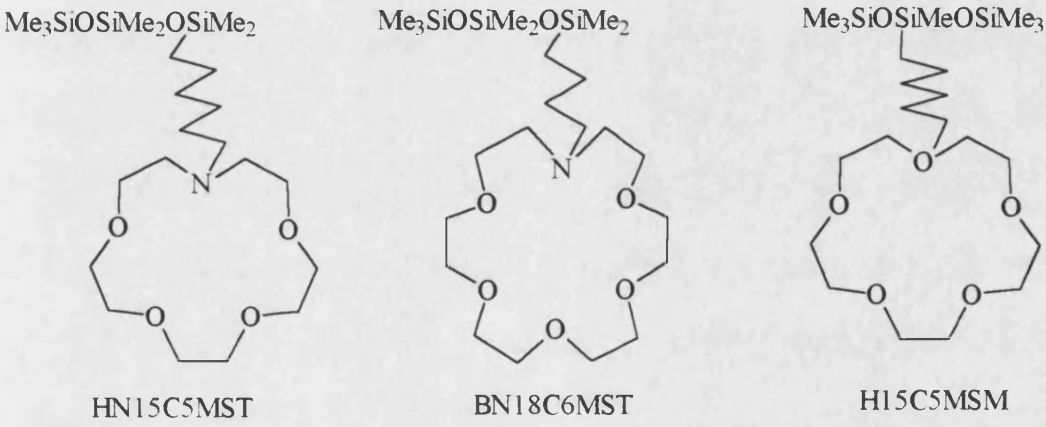


Figure 36

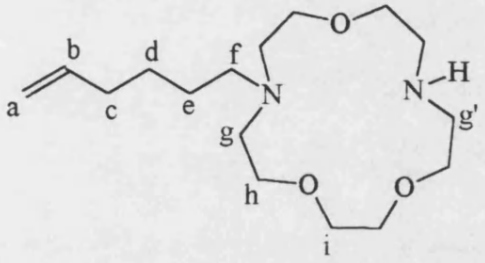
Although these functionalised trisiloxanes were not themselves suitable for use as a liquid extractant, since attachment of the receptor made them soluble in aqueous solvents, their synthesis provided experience in the methodology employed in the hydrosilylation reaction, since the same technique is applicable to the synthesis of functionalised polysiloxane copolymers. The reaction was next carried out on a

(3-4%)-methylhydro-(96-97%)-dimethyl siloxane copolymer, to which was attached oxa- and monoaza-crown ethers.

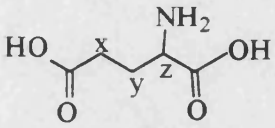
All hydrosilylation reactions were monitored using proton n.m.r spectroscopy with particular attention paid to the vinylic region where the methine proton (~5.8 ppm) and the methylene protons (~5.0ppm) absorb. These resonances were replaced as the reaction occurred by signals due to the two $-CH_2-$ groups α- and β- to the silicon atom which had chemical shifts in the region of 0.0ppm and 0.5ppm respectively. The reaction was also monitored by the disappearance of the Si-H resonance at around 4.8 ppm in the n.m.r. spectrum, and the Si-H stretching vibration at around 2100 cm^{-1} in the infra-red spectrum. In order to prevent cross-linking of the siloxanes caused by hydrolysis of unreacted Si-H residues, a slight excess of the functionalised crown ether material was added to the reaction mixture. The products were purified by removal of the reaction solvent in vacuo, followed by washing with methanol, decanting, and then careful distillation at reduced pressure to remove all remaining traces of unreacted functionalised crown ether and residual solvents. Isolated yields for the model siloxanes ranged from 60% to 73%, and yields for the oxa- and monoaza-crown ether functionalised copolymers were between 51% and 85%. As expected the trisiloxanes containing an end chain functionality (HN15C5MST, BN18C6MST) generally exhibited three ^{29}Si NMR signals in their spectrum, centred at approximately +7 ppm (Me_3SiO- and $OSi(Me_2)CH_2-$) and approximately -21ppm ($OSi(Me_2)-$), whereas the 3-substsituted trisiloxane (H15C5MSM) showed only two ^{29}Si resonances. Silicon NMR spectra of the functionalised siloxane polymers were dominated by absorptions in the -22 ppm region assigned to the repeat $-OSi(Me_2)-$ unit.

4.7.3. Interaction Studies Between Non-Active Mono-Functional Oxa- and Aza-Crown Ethers and GABA or Glutamic Acid

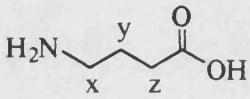
COSY n.m.r. studies were carried out on mixtures of 1-alkenyl functionalised mono- and diaza-crown ethers with either GABA or glutamic acid, as well as on the individual components. The effect of the spacer chain on the aza-crown ether, in particular HDN15C5 and HN18C6, was first evaluated even though the substituent was unlikely to affect the host-guest interaction (**Tables 4.5** to **4.9**).



HDN15C5



DGA



GABA

Proton	δ	δ(HDN15C5/DGA)	Δδ(HDN15C5/DGA)(pH 8)
a	4.84	4.97	+0.13
b	5.76	5.82	+0.08
c	1.92	1.96	+0.04
d	1.20	1.38	+0.18
e	1.32	1.42	+0.10
f	2.36	3.05	+0.69
g	2.59	3.20 (g' 3.00)	+0.61(+0.41)
h	3.50	3.72	+0.22
i	3.50	3.64	+0.14
x	2.34	2.26	+0.12
y	1.94	2.06	+0.12
z	3.60	3.90	+0.30

Table 4.5 ^{1}H N. M. R. Chemical Shift Data for 1:1 HDN15C5 / DGA (pH 8)

Proton	δ	δ(HDN15C5/GABA)	Δδ(HDN15C5/GABA)(pH 9)
a	4.84	4.97	+0.13
b	5.76	5.84	+0.12
c	1.92	2.04	+0.12
d	1.20	1.34	+0.14
e	1.32	1.46	+0.14
f	2.36	2.55	+0.19
g	2.59	2.80 (g' 2.74)	+0.21(+0.15)
h	3.50	3.62	+0.12
i	3.50	3.62	+0.12
x	2.05	2.30	+0.25
y	1.65	1.82	+0.17
z	2.75	2.99	+0.24

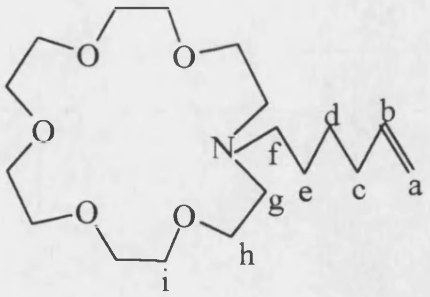
Table 4.6 ^{1}H N. M. R. Chemical Shift Data for 1:1 HDN15C5 / GABA (pH 9)

Proton	δ	δ(HDN15C5/HCl)	Δδ(HDN15C5/HCl)
a	4.84	4.65	-0.19
b	5.76	5.51	-0.25
c	1.92	1.74	-0.18
d	1.20	1.08	-0.12
e	1.32	1.20	-0.12
f	2.36	2.88	+0.52
g	2.59	3.07	+0.48
h	3.50	3.46	-0.04
i	3.50	3.35	-0.15
g'	2.59	2.92	+0.33

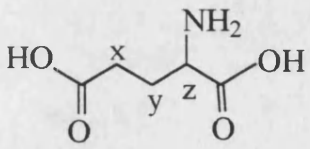
Table 4.7 ^{1}H N. M. R. Chemical Shift Data for 1:1 HDN15C5 / HCl (pH 1)

Proton	Δδ(HDN15C5/HCl)	Δδ(HDN15C5/DGA)	Δδ(HDN15C5/GABA)
a	-0.19	+0.13	+0.13
b	-0.25	+0.08	+0.12
c	-0.18	+0.04	+0.12
d	-0.12	+0.18	+0.14
e	-0.12	+0.10	+0.14
f	+0.52	+0.69	+0.19
g	+0.48	+0.61	+0.21
h	-0.04	+0.22	+0.12
i	-0.15	+0.14	+0.12
g'	-0.33	+0.41	+0.15

Table 4.8 Comparison of the ^{1}H N. M. R. Chemical Shift Data for 1:1 HDN15C5 with DGA, GABA, or excess HCl.



HN18C6



DGA

Proton	δ	δ(HN18C6/DGA)	Δδ(HN18C6/DGA)(pH 6.5)
a	5.10	5.15	+0.05
b	5.98	5.97	-0.01
c	2.18	2.23	+0.05
d	1.47	1.60	+0.13
e	1.58	1.79	+0.21
f	2.62	3.30	+0.68
g	2.88	3.50	+0.62
h	3.75	3.90	+0.15
i	3.75	3.80	+0.05
x	2.34	2.44	+0.10
y	1.94	2.13	+0.19
z	3.60	3.80	+0.20

Table 4.9 ^{1}H N. M. R. Chemical Shift Data for 1:1 HN18C6 / DGA (pH 6.5)

When HDN15C5 or HN18C6 were mixed in solution with a molar equivalent of glutamic acid the protons having the largest change in chemical shift were those on the carbon atoms of the side-chain and the macrocycle α- to the tertiary nitrogen. The shift changes for these protons were in the region of 0.60 ppm (**Tables 4.5** and **4.9**). When HDN15C5 was mixed in a 1:1 ratio with GABA the same α-N-methylene protons of the macrocycle showed the largest chemical shift change (0.2 ppm). When HDN15C5 was made strongly acidic with HCl (**Tables 4.7** and **4.8**) the methylene protons α- to the N atom again showed the greatest shift differences. Thus in all cases the chemical shift changes were mainly centred around

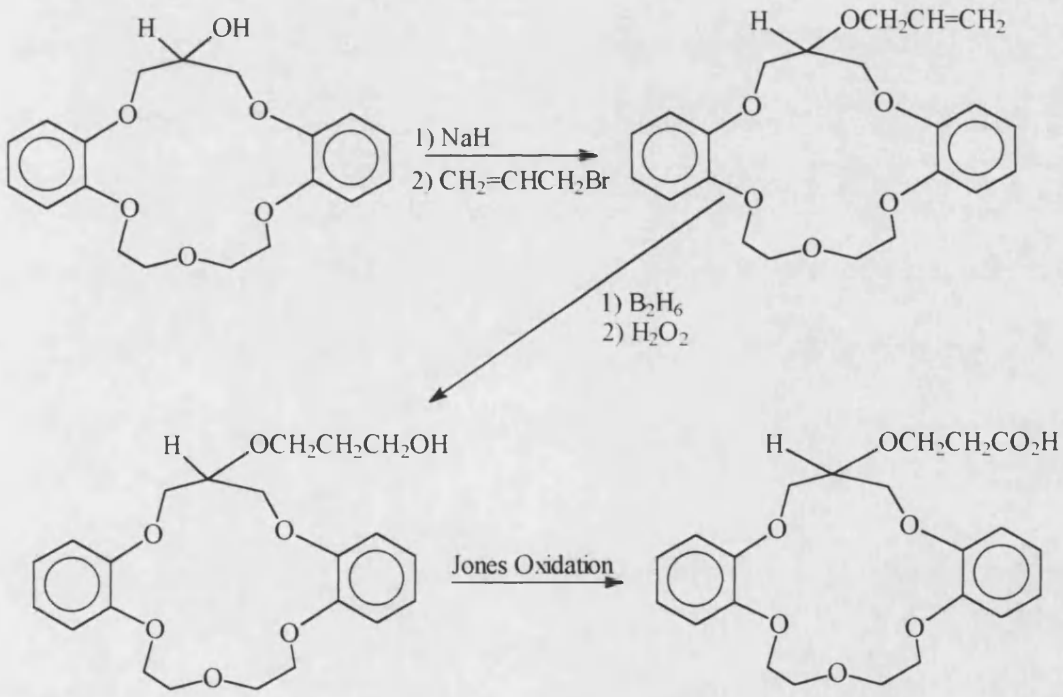
the nitrogen atom of the diaza-crown ether, and are probably caused by protonation of the nitrogen from either the added acid, or from proton transfer from the guest to the host, with glutamic acid protonating the nitrogen centre of the receptor because it is a stronger acid readily than GABA.

The effect of adding a side-chain to an aza-crown ether is most clearly revealed in results obtained on the mono-functionalised diaza-crown ether (HDN15C5) - glutamic acid system, since there are two different nitrogen species present in the macrocycle (**Table 4.5**). The largest changes in chemical shift are noted for the protons on carbon atoms α- to the nitrogen centre, as noted previously. In this instance the larger shift (+0.61 ppm) occurs for the protons (g) on the carbons α- to the tertiary nitrogen, compared to shifts of +0.41 ppm for the protons (g') on the carbon atoms α- to the secondary amine. Although less marked, similar trends occur in the HDN15C5 - GABA system (**Table 4.6**), with the protons g and g' of the macrocycle having shift changes of +0.20 ppm and +0.15 ppm respectively. From these results it can be inferred that the tertiary nitrogen atom is more strongly involved in host-guest interactions than the secondary amine, with these interactions involving proton transfer from the guest onto the tertiary nitrogen centre of the diaza-crown ether. This is in keeping with the general behaviour of amines where tertiary amines are less basic than their analogous secondary amines.

4.8. c) Lariat Azacrown Ethers With Guest-Active Polar Substituents

4.8.1. The Synthesis of Monoaza Crown Ethers with Active Functionalities

The synthetic methodology employed for the preparation of the alkenyl functionalised aza-crown ethers was used to prepare HOLN18C6 and HOICN18C6 which have as terminal functionalities a hydroxyl and a carboxylic acid group respectively. Starting from commercially available 6-bromohexan-1-ol and 6-bromohexanoic acid permitted the preparation of these useful materials which could be isolated from the crude mixture by distillation under reduced pressure in a one-step process. Similar compounds to these have been produced by Bartsch and co-workers[107] to yield a C-functionalised carboxylic acid containing crown ether, an example of which is given in **Scheme 10**.

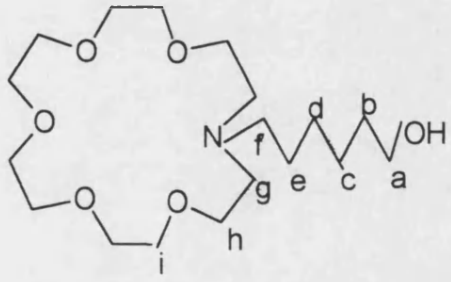


Scheme 10

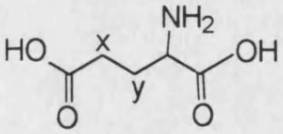
Starting from 3-hydroxy-dibenzo-18-crown-5, which was first synthesised by the reaction of epichlorohydrin with a diphenol compound in aqueous alkaline media, the hydroxyl group of the crown ether was converted to the allyloxy crown ether analogue using sodium hydride and a bromoalkene. The allyloxy group was subsequently modified to a terminal hydroxyl functionality using B_2H_6 and hydrogen peroxide, followed by a Jones oxidation giving the carboxylic acid functionality. A very low yield of 5% was obtained.

4.8.2. Interaction Studies Between a Hydroxyl Terminated Lariat Monoaza-18-crown-6 and GABA and Glutamic Acid

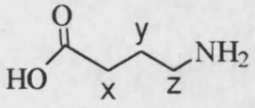
Functionalisation of a mono-aza crown ether with a potentially guest-active side-chain allowed comparison with a non-guest active functionalised mono-aza crown ether. Consequently COSY n.m.r. studies of 1:1 mixtures of HOLN18C6 with GABA and glutamic acid were carried out.



HOLN18C6



DGA



GABA

Proton	δ	δ(HOLN18C6/DGA)	Δδ(HOLN18C6/DGA)(pH 6.5)
a	3.55	3.58	+0.03
b	1.52	1.54	+0.02
c	1.28	1.39	+0.11
d	1.32	1.39	+0.07
e	1.44	1.67	+0.23
f	2.49	3.18	+0.69
g	2.76	3.40	+0.64
h	3.76	3.80	+0.04
i	3.76	3.68	+0.06
x	2.34	2.05	+0.29
y	1.94	2.32	+0.38
z	3.60	3.68	+0.08

Table 4.10 ^{1}H N. M. R. Chemical Shift Data for 1:1 HOLN18C6 / DGA (pH 6.5)

Proton	δ	δ(HOLN18C6/GABA)	Δδ(HOLN18C6/GABA)(pH 10.5)
a	3.55	3.54	-0.01
b	1.52	1.50	-0.02
c	1.28	1.30	+0.02
d	1.32	1.30	-0.01
e	1.44	1.50	+0.06
f	2.49	2.67	+0.18
g	2.76	3.40	+0.64
h	3.76	3.79	+0.03
i	3.76	3.68	-0.08
x	2.05	2.20	+0.15
y	1.65	1.78	+0.13
z	2.75	2.83	+0.08

Table 4.11 ^{1}H N. M. R. Chemical Shift Data for 1:1 HOLN18C6 / GABA (pH 10.5)

Proton	δ	δ(HOLN18C6/GABA)	Δδ(HOLN18C6/GABA)(pH 6)
a	3.55	3.45	-0.10
b	1.52	1.52	0.00
c	1.28	1.27	-0.01
d	1.32	1.27	-0.05
e	1.44	1.41	-0.03
f	2.49	3.09	+0.56
g	2.76	3.27	+0.51
h	3.76	3.68	-0.08
i	3.76	3.55	-0.21
x	2.05	1.97	-0.08
y	1.65	1.57	-0.08
z	2.75	2.69	-0.06

Table 4.12 ^{1}H N. M. R. Chemical Shift Data for 1:1 HOLN18C6 / GABA (pH 6)

Proton	δ	δ(HOLN18C6/HCl)	Δδ(HOLN18C6/HCl)	Δδ(HOLN18C6/DGA)
a	3.55	3.65	+0.10	+0.03
b	1.52	1.56	+0.04	+0.03
c	1.28	1.37	+0.09	+0.11
d	1.32	1.37	+0.05	+0.07
e	1.44	1.63	+0.19	+0.23
f	2.49	3.13	+0.64	+0.69
g	2.76	3.38	+0.62	+0.64
h	3.76	3.75	-0.01	+0.04
i	3.76	3.65	+0.11	+0.06

Table 4.13 Comparison Between the ^{1}H N. M. R. Chemical Shift Data for 1:1 HOLN18C6 with HCl and DGA

By comparing the data in **Tables 4.9** to **4.13** it can be seen that the alkenyl (HN18C6) and hydroxyl (HOLN18C6) terminated aza-crown ethers, when mixed with glutamic acid at pH 6.5, give similar changes in chemical shift for all the protons on both the host and the guest. This indicates that the side-chain hydroxyl group does not enhance significantly the intermolecular hydrogen bonding between the host and guest. Instead proton transfer from the guest onto the nitrogen centre of all the aza-crown ethers appears to occur in preference to other host-guest interactions, as inferred from the large chemical shift changes centred around the nitrogen atom in both HN18C6 and HOLN18C6.

The 1-alkene functionality on an aza-crown ether can be used for two purposes. It may be used to attach the crown ether to a siloxane polymer, or it could be subjected to further synthetic modification, particularly oxidation, to give a polar, and hence potentially guest-active substituent. Selective oxidation of one of the two alkenyl groups on a difunctional diaza-crown ether would allow the potentially guest-active functionalised crown ether product to be tethered to a siloxane via the residual terminal alkene moiety. The synthesis of HDIOLN18C6 and HDIOLHN18C6, by oxidation of the vinyl moiety of HN18C6 and DHDN18C6 respectively, was attempted. Neither $KMnO_4$[113] or iodine / silver acetate[166] were effective in producing more than a minor yield of the required product as judged by n.m.r. spectroscopy. Alternative methods are available for the oxidation of alkenes[201] but they were not attempted since these were also likely to lead to a complex range of reaction products and an alternative route involving the nucleophilic substitution method described earlier[113] was used to form a -diol terminated lariat aza-crown ether (PDIOLN18C6). In view of the non-availability of longer chain n-bromo-1,2-diols and the difficulties encountered in the -diol synthesis, this type of functionalised crown ether was not examined further.

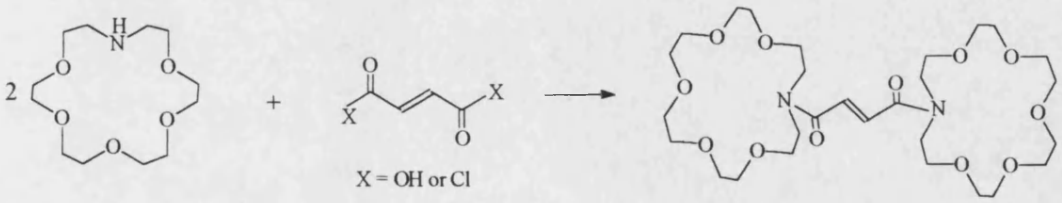
Attempts to isolate solid-state complexes from 1:1 mixtures of aza- and oxa-crown ethers (HN18C6, DHDN18C6, BN15C5 and H15C5) with the simple organic acids and amino-acids were unsuccessful even though a range of solvents, including methanol, dichloromethane, tetrahydrofuran and water and various binary mixtures, were used. It appears likely therefore that the intermolecular interactions between host and guest occurred primarily through proton transfer from the guest onto the host, resulting in the changes in chemical shift noted, and that more effective receptors would require additional hydrogen bonding sites to bind a guest compound effectively enough to permit its removal from the aqueous phase. An effective host

species would need to generate further, and structure specific, hydrogen bonds in preference to, or in addition to, the proton transfer process which appears to occur readily in the N-containing aza-crown ether systems investigated. The use of an additional crown ether moiety was chosen with the aim of producing a "sandwich" type of receptor. This would also allow some guest species to be bonded in more than one orientation, so increasing the likelihood that an effective and selective host-guest complex would result.

4.9. d) Bis (Monoaza-Crown Ethers)

4.9.1. Synthesis of Bis (Monoaza-Crown Ether) Compounds

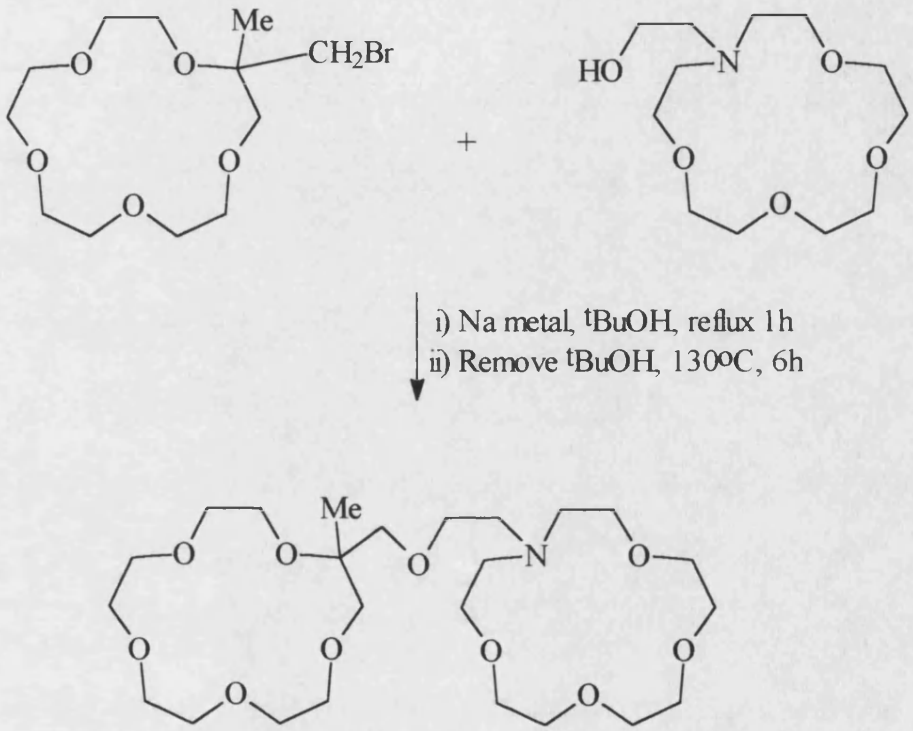
The linked crown ether species were based on two monoaza-18-crown-6 units. Two routes, using either a diacid dichloride or a diacid, were tried for the introduction of a suitable bridge between two monoaza-crown ethers. The linking of the two macrocycles by these types of bridging compounds would, in this instance, yield a diamide. An example of this methodology is shown in **Scheme 11**.



Scheme 11

Other linked crown ether systems have also been reported in the literature. Two benzo-crown ethers have been linked by a diacid bridge by Lui et al[202] using a polyphosphoric acid catalysed reaction. Jeong and Pyun[111] used a diacid dichloride to form the link between two aza-crown ethers. Work by Okahara et al[203] centred

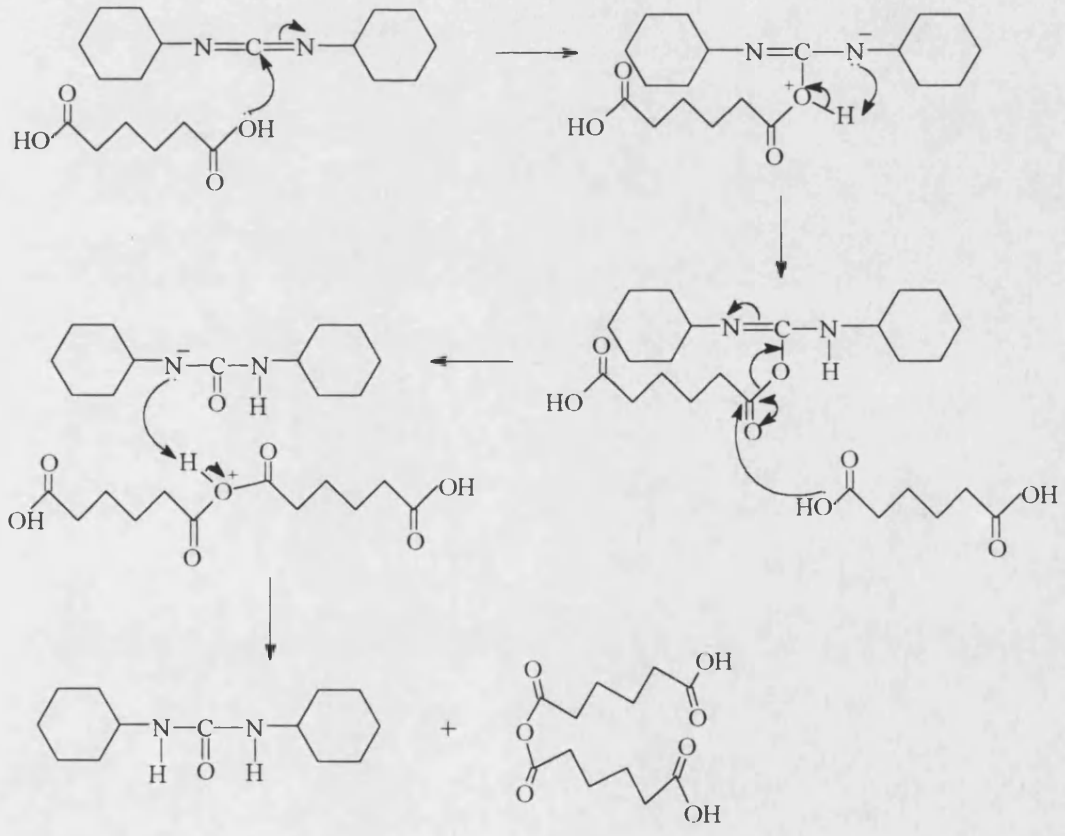
on a bromomethyl functionalised oxa-crown ether and a hydroxyl functionalised aza-crown ether which were joined, via these respective functionalities, using a nucleophilic substitution reaction (**Scheme 12**).



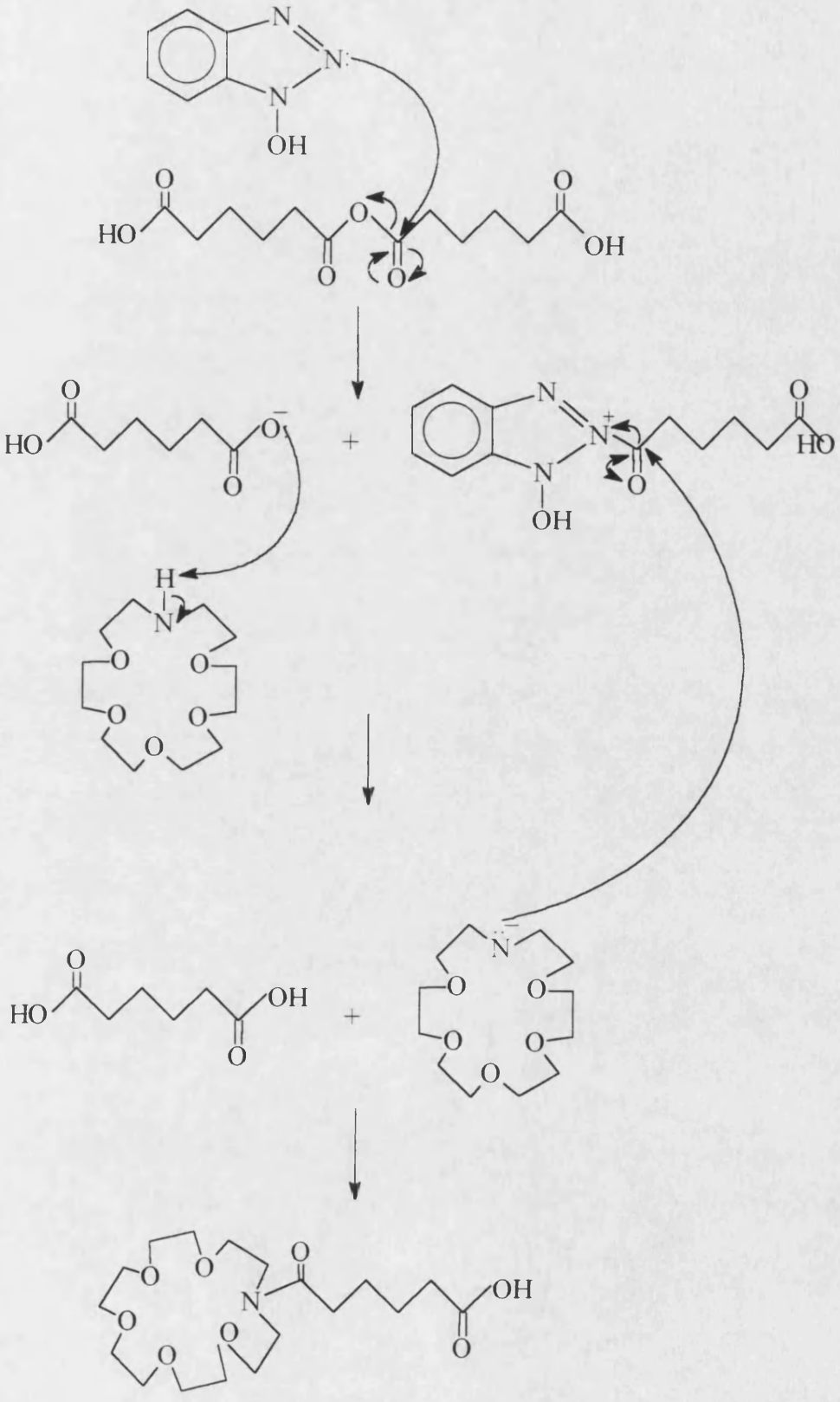
Scheme 12

The preparation of the novel bis(monoaza-crown ethers) (BisN18C6Sub, BisN18C6Muc, BisN18C6Fum, BisN18C6Ita) using the diacid dichloride route was attempted using an extension of the method reported for the functionalisation of aza-crown ethers[113], but it proved largely unsuccessful. Despite ensuring that the solvents, reagents and glassware were all rigorously dried there was evidence, from n.m.r. and I.R. spectroscopies, that only one aza-crown ether moiety had been attached to the linkage and the remaining acid chloride residue had been hydrolysed

to an acid functionality. Therefore an alternative method using dicyclocarbodiimide (DCC) to promote the reaction and hydroxybenzotriazole hydrate (HOBT) as a catalyst was employed. The procedure adopted was based on modifications to published methods[161-162, 204]. The advantage of this reaction, compared to the previous reaction, is that the reagent is less susceptible to moisture and the reaction occurs under mild conditions which will minimise possible degradation of the starting materials and the products. The mode of action of DCC and HOBT is shown in **Schemes 13** and **14**[205] respectively.



Scheme 13



Scheme 14

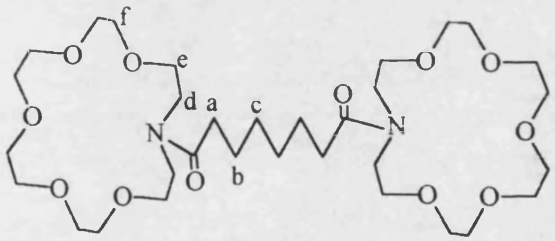
The DCC reacts to form dicyclohexylurea (DCU) which is insoluble in most organic and aqueous solvents and can be almost entirely removed by filtration. The HOBT is regenerated and must therefore be removed, along with any unreacted DCC, during the purification of the product. Removal of these contaminants was achieved by a combination of filtration, column chromatography and distillation at reduced pressure. Full details are given in **Chapter 2**.

Throughout this investigation difficulties were experienced when attempts were made to purify any of the oxa- or aza-crown ether compounds by column chromatography, with many of the products appearing to "stick" on the column, even when using solvent systems which produced suitable retention times in TLC analyses. It was found from empirical studies that partial purification of the crude bis(monoaza crown ether) could be achieved by passing the material through a short neutral alumina column. After collection of the HOBT any material left on the alumina was retrieved by flushing the column with methanol and collecting the washings. Removal of the solvent left only the product and DCC /DCU to be separated by distillation under reduced pressure. The practical method is described more fully in **Chapter 2**. Earlier experiments had shown HOBT is difficult to remove by distillation under reduced pressure and the conditions needed resulted in degradation and charring of the products. The amide group may affect the ability of these bis (monoaza-crown ethers) to form complexes with the target compounds since they now contain a non-basic nitrogen centre. The presence of the amide groups in the bis (monoaza-crown ether) receptors and their effects on the complexing ability of the receptors towards GABA and glutamic acid were investigated by reducing the amide moieties in BisN18C6Iso to amines in BisN18C6Iso(Red). The interactions between these two compounds and the target guest compounds could then be directly compared and the effect of the amide group so determined.

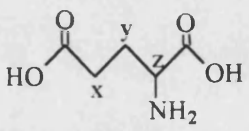
4.9.2. Interaction Studies Between Bis(Monoaza-Crown Ethers) and GABA or Glutamic Acid

The bis-crown ethers synthesised in this study can be divided into two groups, these being a) bis (monoaza-crown ethers) with alkyl and alkenyl bridges and b) bis (monoaza-crown ethers) with aryl bridges. N.m.r. data were recorded on each of BisN18C6Sub, BisN18C6Muc, BisN18C6Fum, BisN18C6Ita, BisN18C6Iso and BisN18C6Iso(Red), and on 1:1 equimolar mixtures of these compounds with either glutamic acid or GABA. These data are given in **Tables 4.14** to **4.38**.

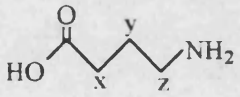
4.9.2.1. a) Interaction Studies Between Alkyl- or Alkenyl-Bridged Bis (Monoaza-Crown Ethers) and GABA or Glutamic Acid



BisN18C6Sub



DGA



GABA

Proton	δ(pH 8.5)	δ(pH 1)	Δδ(pH 8.5/pH 1)
a	2.28	2.00	-0.28
b	1.43	1.17	-0.26
c	1.19	0.92	-0.17
d	3.47	3.27	-0.20
e	3.55	3.48	-0.07
f	3.55	3.48	-0.07

Table 4.14 Proton chemical shifts for BisN18C6Sub at pH 8.5 and pH 1 and the Chemical Shift Difference

Proton	DGA (pH 3.3)	DGA (pH 1.0)	DGA (pH 6.0)
x	2.27	2.35	2.34
y	1.97	1.93	1.94
z	3.65	3.85	3.60

Table 4.15 Proton Chemical Shifts for DGA at pH 1.0, pH 3.3 and pH 6.0

Proton	GABA (pH 6.0)	GABA (pH 1.0)
x	2.05	2.15
y	1.65	1.57
z	2.75	2.68

Table 4.16 Proton Chemical Shifts for GABA at pH 6.0 and pH 1.0

Proton	GABA (pH 1)	GABA/BisN18C6Sub (pH 1)	Δδ GABA (pH 1)
x	2.15	2.25	+0.10
y	1.57	1.68	+0.11
z	2.68	2.78	+0.10

Table 4.17 Differences Between Proton Chemical Shifts for GABA in Individual and 1:1 Mixed Samples at pH 1

Proton	GABA (pH 6)	GABA/BisN18C6Sub (pH 8)	Δδ GABA (pH 8)
x	2.05	2.11	+0.06
y	1.65	1.72	+0.07
z	2.75	2.83	+0.08

Table 4.18 Differences Between Proton Chemical Shifts for GABA in Individual and 1:1 Mixed Samples at pH 6-8

Proton	DGA (pH 1)	DGA/BisN18C6Sub (pH 1)	Δδ DGA (pH 1)
x	2.35	2.33	-0.02
y	1.93	1.93	0.00
z	3.85	3.85	0.00

Table 4.19 Differences Between Proton Chemical Shifts for DGA in Individual and 1:1 Mixed Samples at pH 1

Proton	DGA (pH 6)	DGA/BisN18C6Sub (pH 6)	Δδ DGA (pH 6)
x	2.34	2.37	+0.03
y	1.94	1.98	+0.04
z	3.60	3.50	-0.10

Table 4.20 Differences Between Proton Chemical Shifts for DGA in Individual and 1:1 Mixed Samples at pH 6

Proton	BisN18C6Sub/DGA (pH 1)	BisN18C6Sub/DGA (pH 6)	Δδ(pH 1/pH 6)
a	2.12	2.27	+0.15
b	1.28	1.45	+0.17
c	1.03	1.20	+0.17
d	3.47	3.45	-0.02
e	3.32	3.54	+0.22
f	3.39	3.54	+0.15
x	2.35	2.37	+0.02
y	1.93	1.98	+0.05
z	3.85	3.50	-0.35

Table 4.21 Proton Chemical Shifts and a Comparison of the Shift Between BisN18C6Sub and DGA at pH 1 and pH 6

Proton	BisN18C6Sub/GABA(pH 6)	BisN18C6Sub/GABA(pH 1)	Δδ(pH 1/pH 6)
a	2.26	2.15	-0.11
b	1.43	1.32	-0.11
c	1.18	1.08	-0.10
d	3.44	3.35	-0.09
e	3.56	3.44	-0.12
f	3.56	3.44	-0.12
x	2.15	2.05	+0.10
y	1.57	1.65	+0.08
z	2.68	2.75	+0.07

Table 4.22 Proton Chemical Shifts and a Comparison of the Shift Between BisN18C6Sub and GABA at pH 1 and pH 6

Proton	BisN18C6Sub	BisN18C6Sub/DGA	ΔδBisN18C6Sub
a	2.28	2.27	-0.01
b	1.43	1.45	+0.02
c	1.19	1.20	+0.01
d	3.47	3.45	-0.02
e	3.55	3.54	-0.01
f	3.55	3.54	-0.01

Table 4.23 Proton Chemical Shifts and a Comparison of the Shifts in BisN18C6Sub When mixed With DGA at pH 6

Proton	BisN18C6Sub	BisN18C6Sub/DGA	ΔδBisN18C6Sub
a	2.00	2.18	+0.18
b	1.17	1.34	+0.17
c	0.92	1.09	+0.17
d	3.27	3.53	+0.26
e	3.48	3.38	-0.10
f	3.48	3.45	-0.03

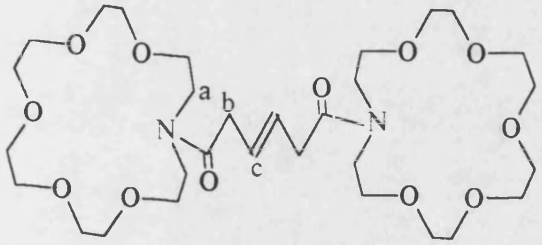
Table 4.24 Proton Chemical Shifts and a Comparison of the Shifts in BisN18C6Sub When mixed With DGA at pH 1

Proton	BisN18C6Sub	BisN18C6Sub/GABA	Δδ BisN18C6Sub
a	2.28	2.26	-0.02
b	1.43	1.43	0.00
c	1.19	1.18	-0.01
d	3.47	3.44	-0.03
e	3.55	3.56	+0.01
f	3.55	3.56	+0.01

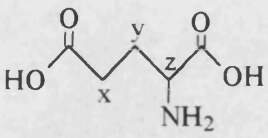
Table 4.25 Proton Chemical Shifts and a Comparison of the Shifts in BisN18C6Sub When Mixed With GABA at pH 8

Proton	BisN18C6Sub	BisN18C6Sub/GABA	Δδ BisN18C6Sub
a	2.00	2.15	+0.15
b	1.17	1.32	+0.15
c	0.92	1.08	+0.16
d	3.27	3.35	+0.08
e	3.48	3.44	-0.04
f	3.48	3.44	-0.04

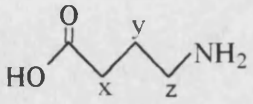
Table 4.26 Proton Chemical Shifts and a Comparison of the Shifts in BisN18C6Sub When Mixed With GABA at pH 1



BisN18C6Muc



DGA



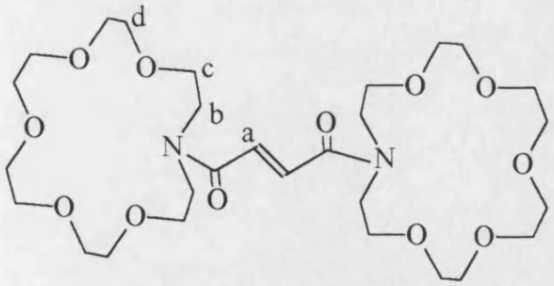
GABA

Proton	δ	δ(BisN18C6Muc/DGA) (pH4)	Δδ
a	3.53	3.53	0.00
b	3.09	3.10	+0.01
c	5.48	5.49	+0.01
x	2.27	2.39	+0.12
y	1.97	1.98	+0.01
z	3.65	3.65	0.00

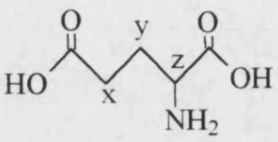
Table 4.27 Differences Between Proton Chemical Shifts in BisN18C6Muc and DGA in Individual and Mixed Samples at pH 4

Proton	δ	δ(BisN18C6Muc/GABA) (pH 6)	Δδ
a	3.53	3.52	-0.01
b	3.09	3.10	+0.01
c	5.48	5.49	+0.01
x	2.05	2.12	+0.07
y	1.65	1.74	+0.09
z	2.75	2.84	+0.09

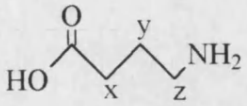
Table 4.28 Differences Between Proton Chemical Shifts in BisN18C6Muc and GABA in Individual and Mixed Samples at pH 6



BisN18C6Fum



DGA



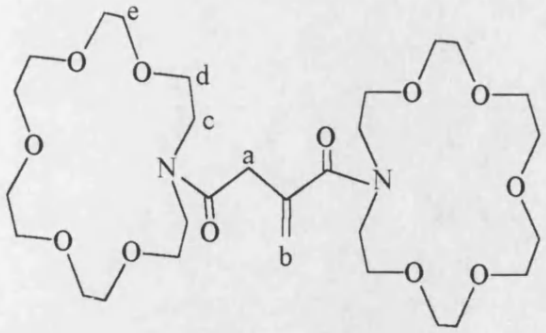
GABA

Proton	δ	δ(BisN18C6Fum/DGA) (pH 6)	Δδ
a	3.64	3.68	+0.04
b	3.53	3.57	+0.04
c	3.53	3.57	+0.04
x	2.34	2.38	+0.04
y	1.94	1.99	+0.05
z	3.60	3.63	+0.03

Table 4.29 Differences Between Proton Chemical Shifts in BisN18C6Fum and DGA in Individual and Mixed Samples at pH 6

Proton	δ	δ(BisN18C6Fum/GABA) (pH 6.5)	Δδ
a	3.64	3.63	-0.01
b	3.53	3.55	+0.02
c	3.53	3.55	+0.02
x	2.05	2.12	+0.07
y	1.65	1.73	+0.08
z	2.75	2.72	-0.03

Table 4.30 Differences Between Proton Chemical Shifts in BisN18C6Fum and GABA in Individual and Mixed Samples at pH 6.5



BisN18C6Ita **DGA** **GABA**

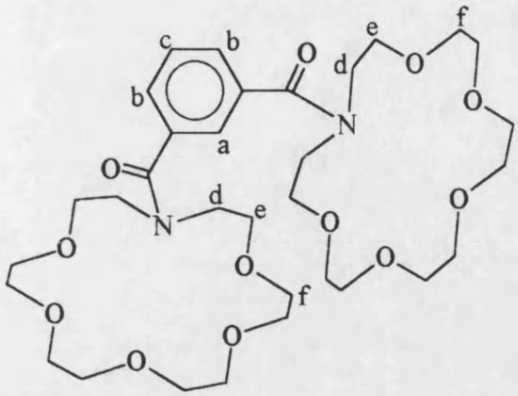
Proton	δ	δ(BisN18C6Ita/DGA) (pH 6)	Δδ
a	1.95	1.90	-0.05
b	6.25	6.39	+0.14
c	3.63	3.63	0.00
d	3.63	3.63	0.00
e	3.63	3.63	0.00
x	2.34	2.41	+0.07
y	1.94	1.99	+0.05
z	3.60	3.65	+0.05

Table 4.31 Differences Between Proton Chemical Shifts in BisN18C6Ita and DGA in Individual and Mixed Samples at pH 6

Proton	δ	δ(BisN18C6Ita/GABA) (pH 6.5)	Δδ
a	1.95	1.90	-0.05
b	6.25	6.39	+0.14
c	3.63	3.63	0.00
d	3.63	3.63	0.00
e	3.63	3.63	0.00
x	2.05	2.08	+0.03
y	1.65	1.72	+0.07
z	2.75	2.83	+0.08

Table 4.32 Differences Between Proton Chemical Shifts in BisN18C6Ita and GABA in Individual and Mixed Samples at pH 6.5

4.9.2.2. b) Interaction Studies Between Aryl-Bridged Bis (Monoaza-Crown Ethers) and GABA or Glutamic Acid



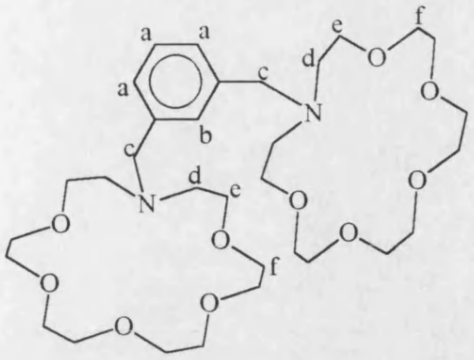
BisN18C6Iso **DGA** **GABA**

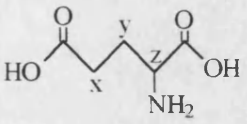
Proton	δ	δ(BisN18C6Iso/DGA) (pH 6)	Δδ
a	7.50	7.49	-0.01
b	7.45	7.41	-0.04
c	7.35	7.36	+0.01
d	3.65	3.65	0.00
e	3.50	3.50	0.00
e	3.50	3.50	0.00
x	2.34	2.40	+0.06
y	1.94	2.00	+0.06
z	3.60	3.68	+0.08

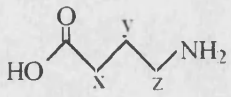
Table 4.33 Differences Between Proton Chemical Shifts in BisN18C6Iso and DGA in Individual and Mixed Samples at pH 6

Proton	δ	δ(BisN18C6Iso/GABA) (pH 6)	Δδ
a	7.50	7.54	+0.04
b	7.45	7.44	-0.01
c	7.35	7.38	+0.03
d	3.65	3.65	0.00
e	3.50	3.50	0.00
e	3.50	3.50	0.00
x	2.05	2.16	+0.11
y	1.65	1.77	+0.12
z	2.75	2.88	+0.13

Table 4.34 Differences Between Proton Chemical Shifts in BisN18C6Iso and GABA in Individual and Mixed Samples at pH 6







BisN18C6Iso(Red) **DGA** **GABA**

Proton	δ (pH 4)	δ(BisN18C6Iso(Red)/DGA) (pH 6)	Δδ
a	7.50	7.48	-0.02
b	7.50	7.48	-0.02
c	4.35	4.40	+0.05
d	3.73	3.72	-0.01
e	3.26	3.28	+0.02
e'	3.34	3.54	-0.02
f	3.54	3.54	0.00
x	2.27	2.38	+0.11
y	1.97	2.00	+0.03
z	3.65	3.63	-0.02

Table 4.35 Differences Between Proton Chemical Shifts in BisN18C6Iso(Red) and DGA in Individual and Mixed Samples at pH 4 and pH 6

Proton	δ (pH 9)	δ(BisN18C6Iso(Red)/DGA) (pH 6)	Δδ
a	7.16	7.48	+0.32
b	7.25	7.48	+0.23
c	3.54	4.40	+0.86
d	2.65	3.72	+1.07
e	3.54	3.28	-0.26
e'	3.54	3.32	-0.22
f	3.54	3.54	0.00
x	2.34	2.38	+0.04
y	1.94	2.00	+0.06
z	3.60	3.63	+0.03

Table 4.36 Differences Between Proton Chemical Shifts in BisN18C6Iso(Red) and DGA in Individual and Mixed Samples at pH 6 and pH 9

Proton	δ (pH 4)	δ(BisN18C6Iso(Red)/GABA) (pH 6)	Δδ
a	7.50	7.50	0.00
b	7.50	7.50	0.00
c	4.35	4.38	+0.03
d	3.73	3.73	0.00
e	3.26	3.29	+0.03
e'	3.34	3.29	-0.05
f	3.54	3.54	0.00
x	2.15	2.21	+0.06
y	1.57	1.77	+0.20
z	2.68	2.87	+0.19

Table 4.37 Differences Between Proton Chemical Shifts in BisN18C6Iso(Red) and GABA in Individual and Mixed Samples at pH 4 and pH 6

Proton	δ (pH 9)	δ(BisN18C6Iso(Red)/GABA) (pH 6)	Δδ
a	7.16	7.50	+0.34
b	7.25	7.50	+0.25
c	3.54	4.38	+0.84
d	2.65	3.73	+1.08
e	3.54	3.29	-0.25
e'	3.54	3.29	-0.25
f	3.54	3.54	0.00
x	2.05	2.21	+0.16
y	1.65	1.77	+0.12
z	2.75	2.87	+0.12

Table 4.38 Differences Between Proton Chemical Shifts in BisN18C6Iso(Red) and GABA in Individual and Mixed Samples at pH 6 and pH 9

The bis (monoaza-crown ether) compounds used in this study, with the exception of BisN18C6Iso(Red), have a tertiary amide moiety rather than a secondary or tertiary amine. The amide nitrogen centres of hosts BisN18C6Sub, BisN18C6Muc, BisN18C6Fum, BisN18C6Ita, BisN18C6Iso are less basic than those of the amine analogue, in this instance BisN18C6Iso(Red), and therefore proton transfer from the guest, or from an added acid, to these amide nitrogen centres does not occur readily. The amide group has some character of an ammonium ion; thus if protonated on nitrogen the resonance stabilisation of the amide would be lost. The protonation of the amide oxygen is favourable however through enhanced resonance stabilisation[206] (**Figure 37**). This is an important consideration in the context of the complexation studies of BisN18C6Iso and BisN18C6Iso(Red) with GABA and DGA which are discussed later

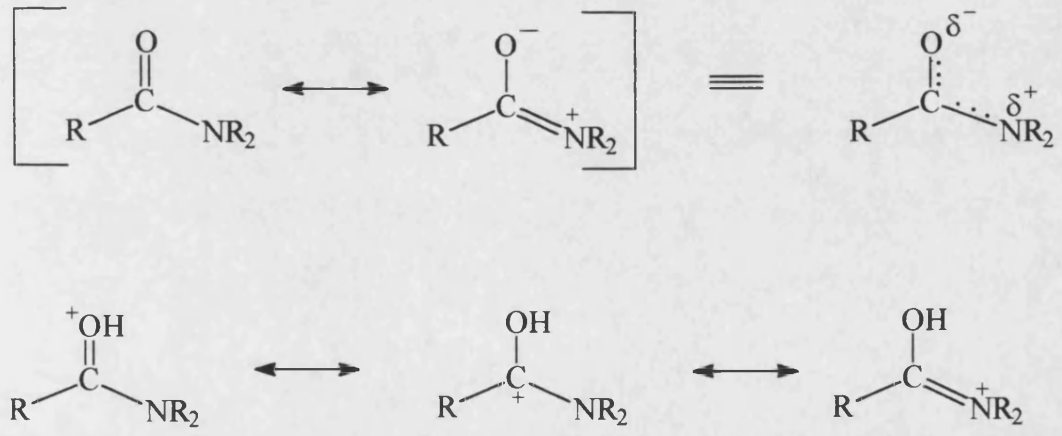


Figure 37

4.9.3. Interaction Studies on BisN18C6Sub-GABA and Glutamic Acid Systems

The bis(monoaza-macrocycles) were prepared in the final stages of this research programme, and consequently their utility in the complexation of the target compounds has yet to be fully explored. The most extensive n.m.r. studies were carried out using BisN18C6Sub, which contains a flexible spacer chain between the two equivalent receptor sites.

In view of the ultimate aim of this work, interaction studies were carried out in D_2O in the pH range 6-8.5. In order to determine how susceptible the amide groups of the host are to protonation, n.m.r. measurements were also carried out on the free host and host / guest combinations in acidified D_2O (pH1). From the data in **Table 4.14** it can be seen that large chemical shifts were noted for the C_a, C_b, C_c and C_d protons in free BisN18C6Sub on acidification, in keeping with protonation of the amide oxygen atom as shown in **Figure 37**. In the pH range 6.0-8.5, the n.m.r. spectra of 1:1 mixtures of this macrocycle with either GABA or DGA are essentially the sum of the spectra of the individual components, indicating no, or very little, interaction. However at pH1 major changes are observed (**Tables 4.21 - 4.26**) for both systems, which cannot be ascribed to protonation of BisN18C6Sub only. Our interpretation is that in acidic solutions, the macrocycle does complex with both GABA and DGA.

Under acidic conditions, the predominant forms of GABA and DGA are those shown below (**Figure 38**). Under strongly acidic conditions the amide oxygen atom of the macrocycle appears to be protonated, which leaves the electronegative oxygen

GABA DGA

Figure 38

atoms of the ring system available for H-bonding interactions with one or more of the functional groups of the guest species. In view of the importance of crown ether-H_3N^+R H-bonding in protonated amine-crown ether chemistry, we suggest that this may also be the most significant part of the interaction between host and guest in the two systems under consideration in this study. A representation of this type of interaction is shown below (**Figure 39**).

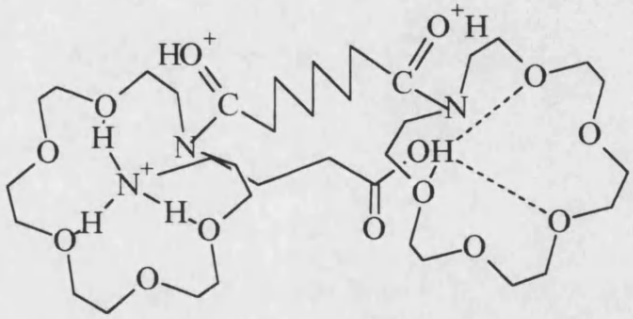


Figure 39

The incorporation of water in one of the rings may enhance the hydrogen bonding effects, as has been noted in **Figure 35**. Attempts to crystallise either the acidified host solution or host - guest combination were not successful.

4.9.4. Interaction Studies on BisN18C6Iso - GABA and Glutamic Acid Systems

The change in chemical shifts at pH6 of the x, y and z protons of the guests GABA and DGA on addition to BisN18C6Iso were +0.11, +0.12, +0.13 ppm for the former (**Table 4.34**) and +0.06, +0.06 and +0.08 ppm for the latter (**Table 4.33**). These indicate that the protons on GABA are now in a slightly different chemical environment which may reflect amino-acid - macrocycle complexation, but the very small changes in the DGA - BisN18C6Iso system are within the limits of experimental error and cannot be interpreted in terms of significant host - guest interactions. Under the conditions of the experiment, GABA would exist predominantly as the zwitter-ion, and BisN18C6Iso would be present as a neutral species. Under these circumstances the crown ether - H_3N^+R interaction may be the only significant factor in determining the chemical shift changes observed (**Figure 40**).

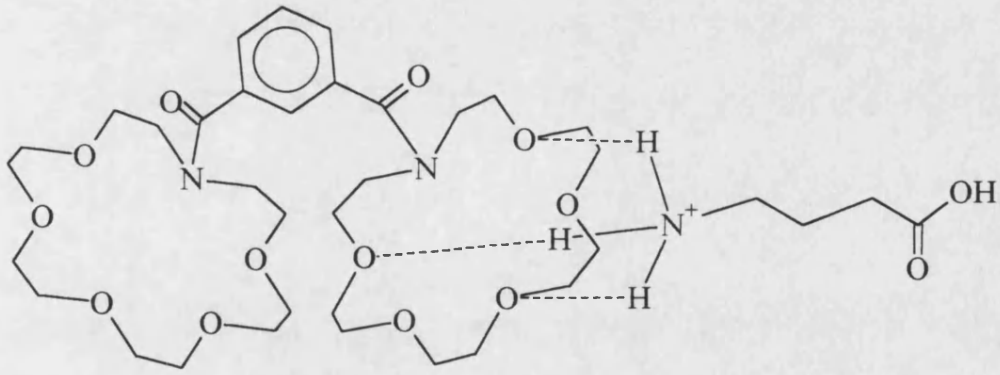
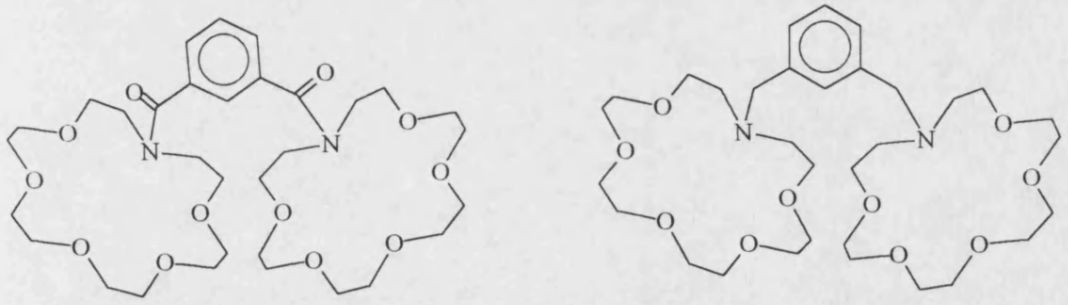


Figure 40

4.9.5. Interaction Studies on BisN18C6Iso(Red) - GABA and Glutamic Acid Systems

Reduction of BisN18C6Iso to BisN18C6Iso(red) resulted in two major changes in the nature if the receptor. Firstly reduction of the amide carbonyl group to a methylene residue will increase the flexibility of the spacer group somewhat, and secondly the receptor now contains two genuine monoaza-18-crown-6 units both with a basic N-centre which can be readily protonated (**Figure 41**).



BisN18C6Iso BisN18C6Iso(Red)

Figure 41

The proton n.m.r. spectrum of BisN18C6Iso(Red) is very sensitive to pH. On changing from pH9 to pH4 chemical shift changes for H_c, H_d and H_e of 0.91, 1.08 and 0.28ppm respectively were observed, consistent with protonation of the receptor in acid conditions. Addition of more aqueous HCl did not change the spectrum further. Large chemical shift differences were also noted for the H_c, H_d and H_e protons of BisN18C6Iso(Red) when the receptor was mixed in solution with equimolar quantities of either GABA or glutamic acid at pH6 (**Tables 4.36. and 4.38.**). Proton transfer is the most likely cause of these changes, and since the spectrum of DGA did not change significantly, there is no positive evidence to indicate complexation is occurring (**Tables 4.35 and 4.36**). However, this is not the case with GABA since there is a change in the proton n.m.r. spectrum for the x, y

and z protons (**Tables 4.37 and 4.38**). From the data tabulated for the proton chemical shift changes for GABA, coupled with the changes noted for the protons of BisN18C6Iso(Red), it can be interpreted that GABA is complexed with the protonated receptor.

4.10. Conclusions

The overall aim of this project was to identify compounds which were able to complex GABA and glutamic acid and to this end a number of mono- and diaza-crown ether based receptors have been synthesised. These range from simple mono-functionalised monoaza-15-crown-5 and monoaza-18-crown-6 through to the diaza-18-crown-6 and ultimately the bis(monoaza-18-crown-6) compounds. These compounds have been produced following the methods given in **Chapter 2.** The functionalised mono- and diaza-crown ethers are readily synthesised, requiring only careful distillation to isolate the product from the crude reaction media. However, the bis(monoaza-crown ethers) are more difficult to isolate, needing a combination of both chromatography and distillation to isolate the product The complexing ability of some of these receptors towards GABA and glutamic acid have been investigated using COSY n.m.r. spectroscopy, the principles and results of which are described in **Chapter 4**.

The early host-guest interaction studies were made on 1: 1 mixtures of N15C5 and valeric acid, glutaric acid and *mono*-methylglutarate. From **Tables 4.1. to 4.4.** it is evident that the protons in N15C5 with the largest change in chemical shift in the presence of an organic acid are those on carbon atoms adjacent (α) to the nitrogen centre of the macrocycle, irrespective of the solvent. These changes are most likely to be due to proton transfer from the carboxylic acid proton of the organic acid onto the nitrogen centre of the N15C5. However, in aqueous solution

there are slight increases in $\Delta\delta$ for the protons on carbons β to the nitrogen atom. These changes may be due to hydrogen bonding between the receptor and a water molecule, in addition to the guest organic acid, since water is readily included within a crown ether ring. The shifts for the α protons on the crown ether are also larger in the aqueous solvent than when compared to those measured in an organic solvent, for identical mixed host-guest systems, (**Tables 4.1. and 4.3**) further suggesting possible inclusion or constructive binding effects of water.

On mixing simple organic guest compounds with monoaza-15-crown-5, the protons most affected (as determined by n.m.r. measurements) were those α to the carboxylate group (**Tables 4.1 to 4.4**). This was apparent for all the organic acids with the α-carboxylate protons of MMG, Val and Glu having chemical shift changes of -0.17, -0.13 and +0.10 ppm respectively. This implies that the acidic end of the guest molecule is active in the interaction with the host as previously stated. The hydroxyl protons, although giving large shift changes in organic solvents, could not be measured in the majority of the analyses due to their readiness to undergo deuterium exchange with the solvent.

When considering the chemical shifts of the lariat functionalised mono-aza crown ethers the effect of the side chain on the guest-complexing ability of the host was first evaluated. The effect of adding a side chain to a macrocycle can be seen most easily from the n.m.r studies on 1:1 mixtures of HDN15C5 and GABA or glutamic acid (**Table 4.5**). HDN15C5 contains both a functionalised (tertiary) and an unfunctionalised (secondary) amine. The largest changes in chemical shift are for the protons α to the nitrogen of HDN15C5. However the largest shift (+0.61 ppm compared to +0.41 ppm) is noted for the protons α to the tertiary nitrogen of HDN15C5 which is the least basic of the two nitrogen centres and will be the more

easily protonated. The protons in the side chain which are α to the nitrogen also have a significant change in shift. This further suggests that the tertiary nitrogen is involved more readily in any interactions which are occurring. Similar results were found for HN18C6 and HOLN18C6, the other functionalised crown ethers studied (**Tables 4.9. to 4.13.**). These chemical shift changes were thought to be due to proton transfer from the guest compound onto the nitrogen of the macrocycle. This theory was tested by acidifying HOLN18C6, to pH 1, with hydrochloric acid and comparing the results with a mixed sample (**Tables 4.7 and 4.8.**). From these results it can be interpreted that proton transfer is the process which is occurring. There were no chemical shift changes noted for either the host or the guest when GABA or glutamic acid were separately mixed with an all oxygen crown ether over a wide pH range suggesting that neither proton transfer, intermolecular binding or protonation from the solvent was taking place.

The all-oxygen receptor does not have any sites which could undergo protonation from either the solvent or from proton transfer from the guest and the absence of chemical shift changes induced in the host when mixed with an organic guest suggests that guest-to-host proton transfer was the mechanism causing the shift changes in the nitrogen containing receptors.

The data for the linked bis(crown ether) systems can be found in **Tables 4.14 to 4.38.** Generally, these differ from the hosts described previously because the 3^{o} amine centre has been replaced by an amide moiety except in BisN18C6Iso(Red) where the two amides have been reduced to amines, which allows a direct comparison of the complexing ability of BisN18C6Iso(red) to be made with its amide analogue BisN18C6Iso. The majority of the n.m.r. studies involved 1:1 mixtures of BisN18C6Sub and GABA and glutamic acid, and from the data it can be inferred that

BisN18C6Sub does indeed complex GABA and glutamic acid in acidic solutions. The COSY data for BisN18C6Iso and BisN18C6Iso(Red) suggests that both of these receptors complex with GABA but do not complex with glutamic acid. Extraction studies on aqueous glutamic acid solutions using a crown ether functionalised polysiloxane copolymer (BN15C5PS) indicated that the functionalised polymer was not capable of extracting the glutamic acid from solution. From the n.m.r. studies on similar monoaza-crown ether (HN18C6) the resulting chemical shift changes are due to proton transfer, and the proton transfer process is likely to be occurring in the glutamic acid-BN15C5PS system. Proton transfer from the glutamic acid onto the BN15C5 results in the glutamic acid not being strongly bound by the monoaza-crown ether. The preferred host-guest interaction would be between the oxygen atoms of the macrocycle and the ammonium cation of the glutamic acid which are known to complex strongly, and it is this latter mode of complexation which is predicted to be occurring for the bis(monoaza-crown ethers) and may indicate that their attachment onto the (3-4%)-methylhydro-(96-97%)-dimethylsiloxane copolymer could result in a material capable of extracting GABA or glutamic acid from solution.

4.11 Further Work

A range of prospective host compounds for the complexation of GABA and glutamic acid have been developed during this study, with the bis (monoaza-crown ether) analogues perhaps offering the most potential since they appear to be useful for the complexation of either of the target guest compounds. Throughout this investigation unsuccessful attempts have been made, using a number of the bis(monoaza-crown ethers) with GABA or glutamic acid, to grow single crystals suitable for X-ray crystallography. Although a wide range of solvents have been utilised endeavouring to grow suitable crystals, there are still a number of solvent mixes could be explored, one of which may yield the necessary environment to produce a crystal. A successful crystal structure would give information on the

orientation of the host donor sites and the size of the cleft which could be expected. Further modification of the receptor to enhance its complexing ability towards a specific compound could then be made using the data from the crystal structure. During this investigation it has been shown that GABA and glutamic acid when mixed with the bis(monoaza-crown ethers) in solution have not been isolable as single crystals. However, due to the importance of empirical structural data in a study of this nature metal cations, in conjunction with a counter-ion such as trifluroacetate or tetrafluoroborate, should be employed since these species have been used in other studies to produce solid-state compounds with crown ether based materials.. The metal cations most expected to produce single crystals with the bis(monoaza-18-crown-6) compounds would be K^+, Sr^+ and Cs^+. It would be hoped that two potassium ions would be included in the complex, one in each of the macrocyclic rings and one strontium or caesium ion could be bound in a sandwich type complex. The presence of an alkenyl functionality would also allow these bis (monoaza-crown ethers) to undergo a hydrosilylation reaction with a polysiloxane resulting in a novel polymeric material. These compounds could then be used in extraction studies to determine whether additional specificity and encapsulation properties have been conferred. The selective extraction of GABA and glutamic acid may not be realised by these new receptors when as free compounds or when attached to a siloxane copolymer but they may mask these target molecules sufficiently to enable the required analytical determinations on fermentation broths to be undertaken. Further complexation studies between the bis(monoaza-crown ethers) and GABA and glutamic acid over a wide pH range should be studied using COSY n.m.r. spectroscopy since this may also prove useful in highlighting the pH at which the host-guest complexation is at a maximum. This is particularly applicable to BisN18C6Iso and BisN18C6Iso(Red) with GABA or glutamic acid where, due to time constraints, only a preliminary study has been undertaken.

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Appendix One

Method: C:\HPCHEM\1\METHODS\AMINOA5.M

Method Information

Modified Amino Acid derivatisation method
Vial 1 Water,Vial 2 OPA, Vial 3 FMOC, Vial 4 Borate Buffer
Column Beckman Ultrasphere 4.6mm x 250mm
Solvent A 20mM CH3COONa, 0.018% TEA, pH 7.2 (CH3COOH), 0.3% THF
Solvent B 20% 100mM CH3COONa pH 7.2 (CH3COOH), 40% CH3CN, 40% MeOH
Flow 2ml/min

LC 1090

Pump (DR5):

Stop Time	25.00 min
Post Time	4.00 min
Flow	2.000 ml/min
Min. Pressure	1 bar
Max. Pressure	400 bar
Oven Temperature	40.0 Centigrade
Solvent A	100.0 % (SOLV A)
Solvent B	0.0 % (SOLV B)
Solvent C	Off (CH3CN)

Injector Program:

Draw Speed	41.7 ul/min
Eject Speed	41.7 ul/min
Mix Speed	41.7 ul/min
Hold after Draw & Eject	0 sec

Line	Function					
1	DRAW	5.0	ul	from	VIAL 3	
2	DRAW	1.0	ul	from	VIAL 1	
3	DRAW	0.0	ul	from	VIAL 0	
4	DRAW	0.5	ul	from	SAMPLE	
5	DRAW	0.0	ul	from	VIAL 0	
6	MIX	6.5	ul	in	LOOP	cycles: 6
7	DRAW	1.0	ul	from	VIAL 2	
8	DRAW	0.0	ul	from	VIAL 0	
9	MIX	7.5	ul	in	LOOP	cycles: 3
10	INJECT					

Contacts:

Contact 1	Off
Contact 2	Off
Contact 3	Off
Contact 4	Off
Column Switch	Deactivated

Method: C:\HPCHEM\1\METHODS\AMINOA5.M

Time Table:

Time [min]	%B	%C	Flow [ml/min]
17.00	60.0		2.000
18.00	100.0		2.000
18.10	100.0		2.000
18.50	100.0		2.500
23.90	100.0		2.500
24.00	100.0		2.000
24.10	100.0		2.000
25.00	0.0		0.450

DIODE ARRAY DETECTOR

Settings:

Stop Time	as Pump: 25.00 min
Post Time	Off
Peakwidth	0.040 min
Sampling Interval	0.160 sec
Autobalance	On

Signals:

	Sample,Bw	Reference,Bw	[nm]
A:	338 10	390 20	
B:	262 16	324 8	
C:	Off		
D:	Off		
E:	Off		
F:	Off		
G:	Off		
H:	Off		

Spectrum:

Store	None
From	200 nm
To	450 nm
Step	2 nm
Threshold	0.5 mAU

Time Table is empty.

Method: C:\HPCHEM\1\METHODS\AMINOA5.M

Specify Report

Destination: Printer
Report Style: Chrom+Short
Quantitative Results sorted by: Signal
Chromatogram Orientation: Portrait

	Size	Min Value	Max Value
Time	1.00		
Response	0.40		

Plot Annotations: Retention Time
Multi Chromatograms: Each in full Scale, Separated

Method: C:\HPCHEM\1\METHODS\AMINOA7.M

Method Information

Calibration Method:D-Glutamic Acid using AMINOA4.M

Run Time Checklist

Pre-Run Cmd/Macro:	on
Name:	LCWASH ON,2,WAIT
Data Acquisition:	on
Standard Data Analysis:	on
Customized Data Analysis:	off
Save GLP Data:	off
Post-Run Cmd/Macro:	off
Save Method with Data:	off

LC 1090

Pump (DR5):

Stop Time	25.00 min
Post Time	4.00 min
Flow	1.000 ml/min
Min. Pressure	1 bar
Max. Pressure	400 bar
Oven Temperature	40.0 Centigrade
Solvent A	100.0 % (SOLV A)
Solvent B	0.0 % (SOLV B)
Solvent C	0.0 % (CH3CN)

Injector Program:

Draw Speed	41.7 ul/min
Eject Speed	41.7 ul/min
Mix Speed	41.7 ul/min
Hold after Draw & Eject	0 sec

Line	Function					
1	DRAW	5.0	ul	from	VIAL 3	
2	DRAW	0.0	ul	from	VIAL 0	
3	DRAW	2.0	ul	from	VIAL 1	
4	DRAW	0.0	ul	from	VIAL 0	
5	DRAW	2.0	ul	from	SAMPLE	
6	DRAW	0.0	ul	from	VIAL 0	
7	MIX	9.0	ul	in	LOOP	cycles: 6
8	INJECT					

Method: C:\HPCHEM\1\METHODS\AMINOA7.M

Contacts:

Contact 1	Off
Contact 2	Off
Contact 3	Off
Contact 4	Off
Column Switch	Deactivated

Time Table:

Time [min]	%B	%C	Flow [ml/min]
17.00	60.0	0.0	1.000
18.00	100.0	0.0	1.000
18.10	100.0	0.0	1.000
18.50	100.0	0.0	2.000
23.90	100.0	0.0	2.000
24.00	100.0	0.0	1.000
24.10	0.0	0.0	1.000

DIODE ARRAY DETECTOR

Settings:

Stop Time	as Pump: 25.00 min
Post Time	10.00 min
Peakwidth	0.040 min
Sampling Interval	0.160 sec
Autobalance	On

Signals:

	Sample,Bw	Reference,Bw	[nm]
A:	338 10	390 20	
B:	Off		
C:	Off		
D:	Off		
E:	Off		
F:	Off		
G:	Off		
H:	Off		

Spectrum:

Store	None
From	200 nm
To	450 nm
Step	2 nm
Threshold	0.5 mAU

Time Table is empty.

Method: C:\HPCHEM\1\METHODS\AMINOA7.M

Sequence Recalibration Table

Cal. Line	Cal. Level	Update Response Factor	Update Retention Times	Recalib Interval

Method: C:\HPCHEM\1\METHODS\AMINOA7.M

Integration Event table "Event"

Event	Value	Time
Initial Area Reject	1.000	Initial
Initial Threshold	-2.000	Initial
Initial Peak Width	0.040	Initial
Initial Shoulders	OFF	Initial

Integration Event table "Event_DAD1A"

Event	Value	Time
Initial Area Reject	1.000	Initial
Initial Threshold	-2.000	Initial
Initial Peak Width	0.040	Initial
Initial Shoulders	OFF	Initial

Specify Report

Destination: Printer
Report Style: Detail+Spectrum
Quantitative Results sorted by: Signal
Chromatogram Orientation: Portrait

	Size	Min Value	Max Value
Time	1.00		
Response	0.40		

Plot Annotations: Retention Time
Multi Chromatograms: Each in full Scale, Separated

Calibration Table

D-Glutamic Acid

Calib. Data Modified : 20 April 1994 10:47:44

Calculate : Area Percent

Rel. Reference Window : 5.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.000 min
Default Multiplier : 1.000000 (if not set in sample table)
Default Sample Amount : 0.000000 (if not set in sample table)
Uncalibrated Peaks : not reported
Partial Calibration : identified peaks calibrated
RT of non-identified peaks not updated

Curve Type : Logarithmic (different for some peaks)
Origin : Included (different for some peaks)

Method: C:\HPCHEM\1\METHODS\AMINOA7.M

Response Factor Update: Average Response Factors of all calibrations
Retention Time Update : Floating Average of Retention Times, New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Signal 1 : DAD1 A, Sig=338,10 Ref=390,20

RT [min]	Sig	Lvl	Amount [ng/ul]	Area	Amt/Area	Ref	Grp	Name
2.929	1	10	50.15000	6.59046e-1	76.09484			D-Glutamic Acid
		11	50.15000	6.62967e-1	75.64479			
		7	100.30000	8.13540e-1	123.28835			
		9	100.30000	7.48332e-1	134.03142			
		8	100.30000	7.91326e-1	126.74929			
		4	501.50000	9.24439e-1	542.49117			
		6	501.50000	9.01724e-1	556.15688			
		5	501.50000	9.03447e-1	555.09622			
		1	1003.00000	9.30123e-1	1078.35204			
		3	1003.00000	1.01931	983.99898			
		2	1003.00000	9.56037e-1	1049.12259			

==========

Method: C:\HPCHEM\1\METHODS\WASH.M

Method Information

Wash Method

Run Time Checklist

Pre-Run Cmd/Macro:	off
Data Acquisition:	on
Standard Data Analysis:	on
Customized Data Analysis:	off
Save GLP Data:	off
Post-Run Cmd/Macro:	off
Save Method with Data:	off

LC 1090

Pump (DR5):

Stop Time	120.00 min
Post Time	4.00 min
Flow	0.500 ml/min
Min. Pressure	1 bar
Max. Pressure	400 bar
Oven Temperature	40.0 Centigrade
Solvent A	0.0 % (SOLV A)
Solvent B	0.0 % (SOLV B)
Solvent C	100.0 % (CH3CN)

Injector Program:

Draw Speed	41.7 ul/min
Eject Speed	41.7 ul/min
Mix Speed	41.7 ul/min
Hold after Draw & Eject	0 sec

Line	Function					
1	DRAW	5.0	ul	from	VIAL 3	
2	DRAW	1.0	ul	from	VIAL 1	
3	DRAW	0.0	ul	from	VIAL 0	
4	DRAW	1.0	ul	from	SAMPLE	
5	DRAW	0.0	ul	from	VIAL 0	
6	MIX	7.0	ul	in	LOOP	cycles: 6
7	DRAW	1.0	ul	from	VIAL 2	
8	DRAW	0.0	ul	from	VIAL 0	
9	MIX	8.0	ul	in	LOOP	cycles: 3
10	INJECT					

Method: C:\HPCHEM\1\METHODS\WASH.M

Contacts:

Contact 1	Off
Contact 2	Off
Contact 3	Off
Contact 4	Off
Column Switch	Deactivated

Pump Time Table is empty.

DIODE ARRAY DETECTOR

Settings:

Stop Time	as Pump: 120.00 min
Post Time	Off
Peakwidth	0.040 min
Sampling Interval	0.160 sec
Autobalance	On

Signals:

	Sample,Bw	Reference,Bw	[nm]
A:	338 10	390 20	
B:	262 16	324 8	
C:	Off		
D:	Off		
E:	Off		
F:	Off		
G:	Off		
H:	Off		

Spectrum:

Store	None
From	200 nm
To	450 nm
Step	2 nm
Threshold	0.5 mAU

Time Table is empty.

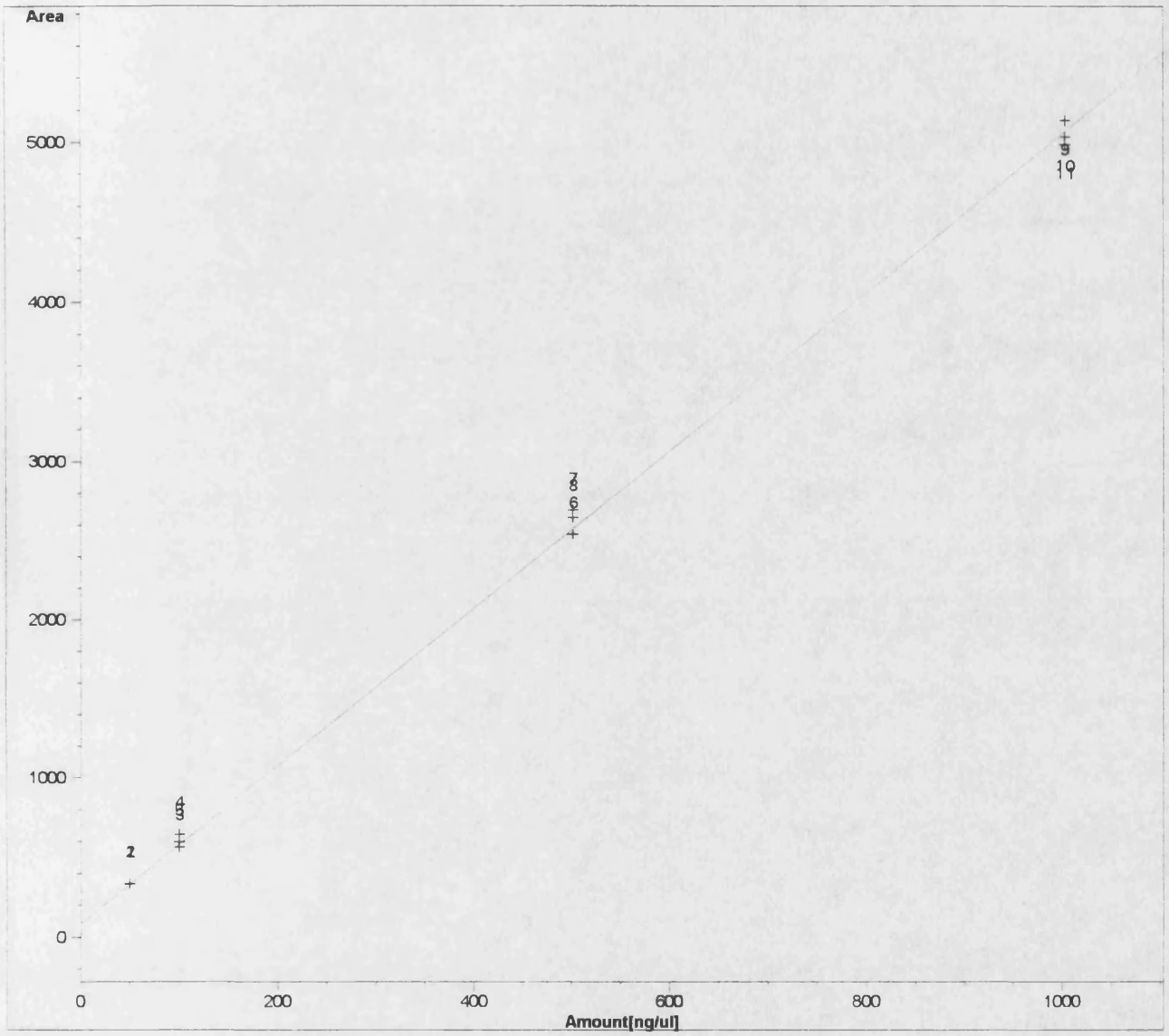
Sequence Recalibration Table

Cal. Line	Cal. Level	Update Response Factor	Update Retention Times	Recalib Interval

Appendix Two

Method C:\HPCHEM\1\METHODS\CALIB4.M

Calibration Curve



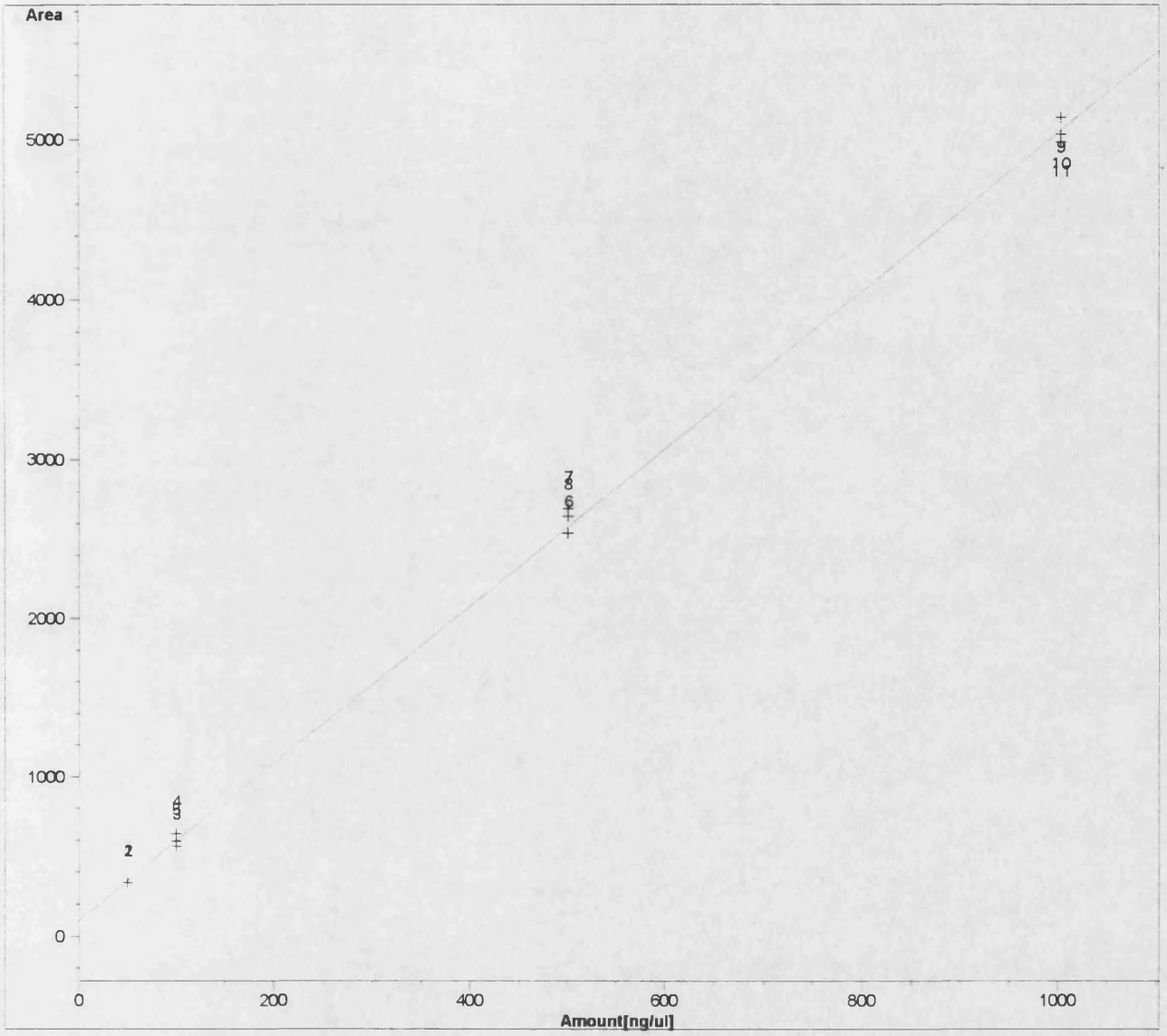
D-Glutamic Acid at exp. RT: 6.160
DAD1 A, Sig=338,10 Ref=390,20
Correlation: 0.99952
Residual Std. Dev.: 65.33155
Formula: y = mx + b
m: 4.96981
b: 88.30265
x: Amount
y: Area

Method C:\HPCHEM\1\METHODS\CALIB4.M

==========

Calibration Curve

==========



D-Glutamic Acid at exp. RT: 6.160
DAD1 A, Sig=338,10 Ref=390,20
Correlation: 0.99958
Residual Std. Dev.: 60.74561
Formula: y = mx + b
m: 4.94533
b: 107.26580
x: Amount
y: Area

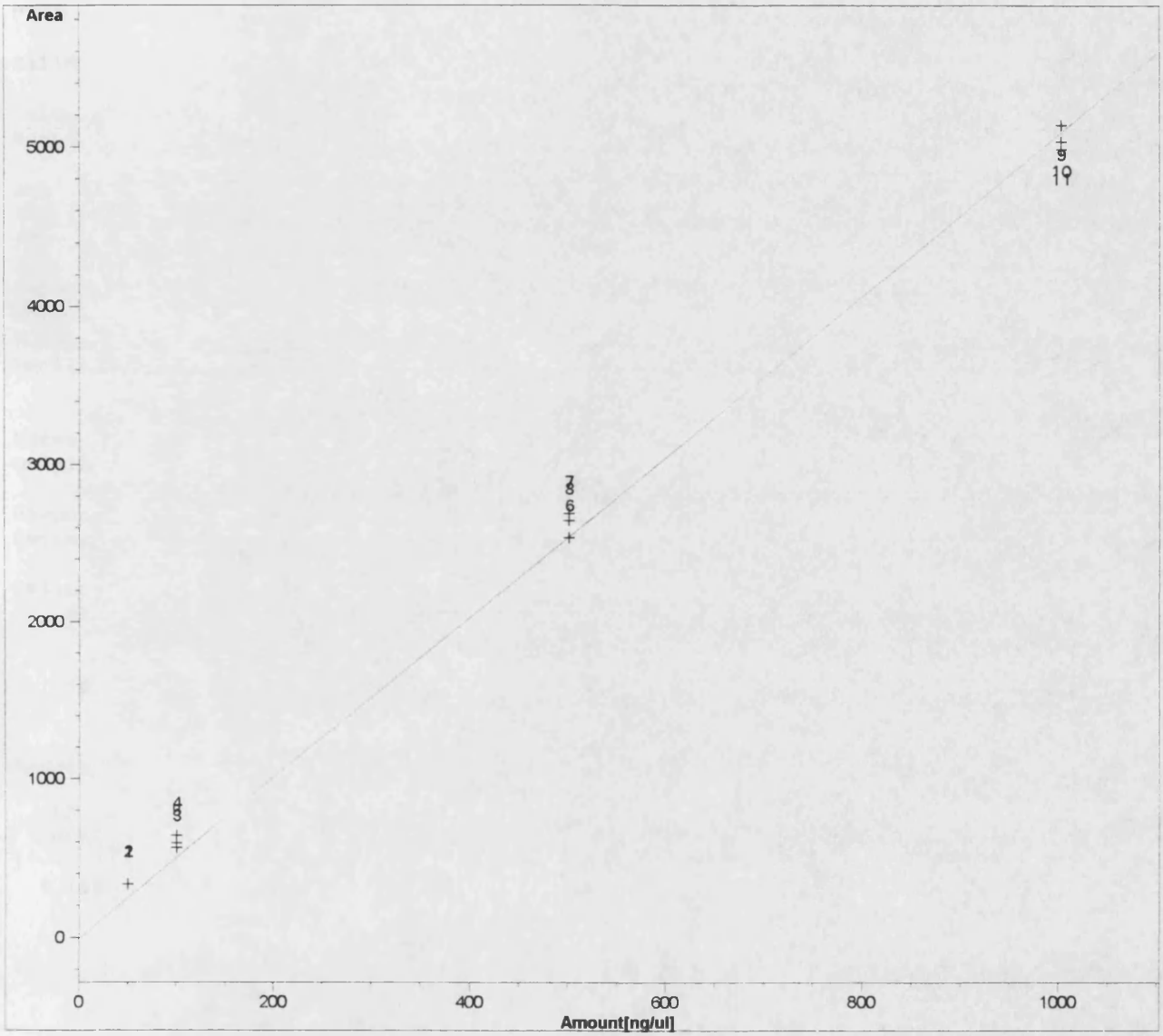
==========

Method C:\HPCHEM\1\METHODS\CALIB4.M

==========

Calibration Curve

==========



D-Glutamic Acid at exp. RT: 6.160
DAD1 A, Sig=338,10 Ref=390,20
Correlation: 0.99956
Residual Std. Dev.: 93.16017
Formula: y = mx
m: 5.08378
x: Amount
y: Area

==========

Method C:\HPCHEM\1\METHODS\CALIB4.M

Calibration Table

D-Glutamic Acid Calibration

Calib. Data Modified : 21 April 1994 15:10:42

Calculate : External Standard
Based on : Peak Areas

Rel. Reference Window : 10.000 %
Abs. Reference Window : 6.000 min
Rel. Non-ref. Window : 5.000 %
Abs. Non-ref. Window : 0.000 min
Default Multiplier : 1.000000 (if not set in sample table)
Default Sample Amount : 0.000000 (if not set in sample table)
Uncalibrated Peaks : not reported
Partial Calibration : identified peaks calibrated
RT of non-identified peaks not updated

Curve Type : Linear (different for some peaks)
Origin : Included (different for some peaks)

Response Factor Update: Average Response Factors of all calibrations
Retention Time Update : Floating Average of Retention Times, New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Signal 1 : DAD1 A, Sig=338,10 Ref=390,20

RT [min]	Sig	Lvl	Amount [ng/ul]	Area	Amt/Area	Ref	Grp	Name
6.160	1	1	50.15000	332.83578	1.50675e-1			D-Glutamic Acid
		2	50.15000	334.10812	1.50101e-1			
		4	100.30000	640.93719	1.56490e-1			
		3	100.30000	562.04523	1.78455e-1			
		5	100.30000	592.89758	1.69169e-1			
		7	501.50000	2689.94824	1.86435e-1			
		6	501.50000	2536.70557	1.97697e-1			
		8	501.50000	2643.39258	1.89718e-1			
		9	1003.00000	5135.39160	1.95311e-1			
		11	1003.00000	4983.99414	2.01244e-1			
		10	1003.00000	5032.50488	1.99304e-1			